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The One Health approach in the context of public health

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The One Health approach in the context of public health

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Editorial: The One Health approach in the context of public health

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Editorial on the Research Topic
The One Health approach in the context of public health

One Health recognizes the interdependence of the health of humans, domestic and wild animals, plants, and environmental ecosystems (1). Frontiers in Public Health recently published 11 articles on this Research Topic representing different aspects of this broad field. This approach mobilizes multiple sectors, disciplines, and communities at varying levels of society to work together, foster wellbeing and tackle threats. It also addresses the collective need for clean water-energy-air, safe and nutritious food, appropriate action on climate changes, and contributing to sustainable development. One Health (whose concepts date at least to the nineteenth century) is not new, but its application is increasingly critical. Our planet is being relentlessly altered by changes in climate and land use, including deforestation, industrialization, and intensive farming practices. Close contact with animals and their environments allows diseases to transmit from animals to humans. Disruptions in environmental conditions and habitats can also provide new pathways for diseases to pass to animals. The mobilization of people, animals, and animal products has increased significantly due to international trade allowing diseases to spread rapidly across the borders and around the globe. It is essential to interconnect these disciplines from regional to global levels, which will establish the pathway to meet the 17 sustainable development goals (SDGs) addressing these public health issues by 2030.

Some serological evidence were observed by several researchers regarding the transmission of viruses from animals to humans with minimal adaptation, but the interface between animals and humans is not much evident (2). Furthermore, human noroviruses have been detected from animal stool samples (3), and using the animal model experimental settings, the cross-species barrier capability was also confirmed by De Graaf et al. (4). The Global Consortium on Climate and Health Education surveyed 160 institutions to understand the stages of climate-health curricula for health professions. Shea et al. (5) concluded that the educational programs vary considerably between institutions and that most responders faced relevant challenges when trying to implement curricular changes in their institutions. Numerous processes were utilized to interlink the environment and human health, and coordinate with unknown threats like climate crisis, environmental pollution, and lack of biodiversity (6). O'Callaghan-Gordo et al. outlined the elements of an online Master's degree in planetary health, complementing a large number of existing post graduate courses in One Health. They also discussed the risk of confusion arising via the proliferation of subtly different approaches to health in the Anthropocene (a proposed name for our new era, in which humans are recognized as a force of planetary transformation).

Knowledge of the diversity and routes of transmission of pathogens is constantly expanding. For example, noroviruses are highly contagious, and infect a wide range of target but not limited to livestock animals, pets, marine mammals, rodents, and humans (7). Furthermore, a low concentration (10–18 virus particles) of this virus can cause severe infection (8). Zheng et al. (9) and Alam et al. (10) demonstrated that the human infections are mostly caused by GI, GII, GIV, GVIII, and GIX, although GII is the frequently detected genogroup worldwide. Using the One Health principles, Yasir et al. also identified the norovirus associated risk factors. The prevalence data reported by Yasir et al. was within the range of other study areas with similar climate, regional conditions, and food habits. Bovine norovirus has been identified as one of the possible etiologies of calf diarrhea (11) causing severe life-threatening effects on the neonatal calf (12). Due to the ever-increasing trade in the livestock industry, new and existing diseases are challenging for the industries. In addition to the microbial threat to the risk factors, Aslam et al. addressed that poor sanitation, unsanitary slaughterhouses, transportation of animals, nomadic lifestyle, and unskilled trained livestock and medical care professionals could be possible parameters to these risk factors and causes for spreading the Crimean-Congo hemorrhagic fever (CCHF). They also identified that the virus pathogenesis could be linked to either destroying cells directly through proliferation or damaging cells indirectly through the release of cytotoxic chemicals.

In separate studies, Ali and Alsayeqh and Bintsis (13) focused on pathogens such as *Escherichia coli*, *Salmonella*, *Campylobacter* (Figure 1), *Shigella*, *Listeria monocytogenes*, and others as well as toxins produced by *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium* species with an emphasis on their animal sources and prevention measures. These pathogens can cause gastrointestinal diseases with a focus on the mechanisms of toxigenic and invasive diarrhea (15). A need for a comprehensive approach to prevent and manage meat-borne diseases from production to consumption was identified by González et al. (16), which is consistent with a One Health approach, recognizing the interconnection between human, animal and environmental health (17). Furthermore, the aerosol transmissibility of intracellular pathogen *Coxiella burnetii* can cause Q fever, a meat-sourced bacterial infection, whose disease burden has been greatly lessened by a commercially available vaccine (18).

The diversity of toxins, microbial resistance genes, and infectious diseases is extremely challenging to deal with because of their propagation natures. Scientists and project administrations are facing numerous complex challenges implementing a sustainable and effective antimicrobial stewardship (AMS) program; furthermore, it is essential to understand these challenges to tailor the stewardship initiatives according to the unique requirements (19), as discussed above. Hassan et al. identified several key issues that impede the implementation of the AMS program in their study areas. Those deficiencies led to well-structured monitoring, evaluation processes, and feedback provision below their standard limits. The World Health Organization (WHO) urged the healthcare organizations in developing and underdeveloped communities to conduct strategic planning and implementation toward the antimicrobial resistance action plan to standardize and overcome the challenges during the implementation of the AMS program. Pattnaik et al. performed an 86-item questionnaire to collect data consisting of semi-open questions based on socio-demographic characteristics, antibiotics usage, awareness of antimicrobial resistance, and healthcare utilization and quality of life (WHO-QOL BREF scale) in India. The findings from their study are very similar to those of Jordan and Nepal. It is essential to have knowledge on the proper antimicrobial use. Nearly 1/7th of the participants preferred taking antimicrobial medicines without prescription over the counter or without the consultation with healthcare workers, which is a very common scenario among the consumers in most of the developing, and underdeveloped countries.

Under the umbrella of One Health, individual, population, and ecosystem health are interconnected. To apply a species-spanning approach to medicine in One Health initiative, it is essential to incorporate not only contagious (infections) or other types of diseases (e.g., cancer or metabolic diseases), but also mental health and anything from behavioral problems related to addictions or depressions (20). Yun et al. identified that sleep deprivation may be one of the crucial factors governing the association of quick return (QR) to work with sleep disturbances (SD) and depressive symptoms (DS). The QR could shorten the duration of sleep, and cause disrupted and restless sleep (most found in the shift-work system) (21, 22). Yun et al. identified in their mediation analysis regardless of demographic factors and the working environment that the SD originating from QR could form a mediator associating QR and depression, which indicates that QR and DS are associated with each other. Following the One Health approach, numerous studies have been conducted to isolate and identify the proper drug molecules from natural sources to cure simple to complex diseases.

Arisaema jacquemontii Blume, a medicinal plant, treats several life-threatening diseases including viral infections because of its antioxidant, anti-cancerous, antimalarial, anti-vermicidal, and antiviral properties. Shehzadi et al. isolated and characterized (molecular docking) an antiviral COVID-19 protein [protease (6LU7)], from *Arisaema jacquemontii* Blume which has a maximum binding affinity of 8.1 kJ/mol and hydrogen bonding interactions. This group successfully concluded that this molecule (6LU7) could be useful to develop more phytochemical-based COVID-19 therapeutics. Rather and Mohammad (23) and Yang et al. (24) reported on several plant derived active ingredients including carotenoids, sterols, aliphatic compounds, monoterpenes and sesquiterpenes, triterpenoids, and others effectively. The plant-originated chemicals contain secondary metabolites, which could be effective drug molecules with several promising properties,

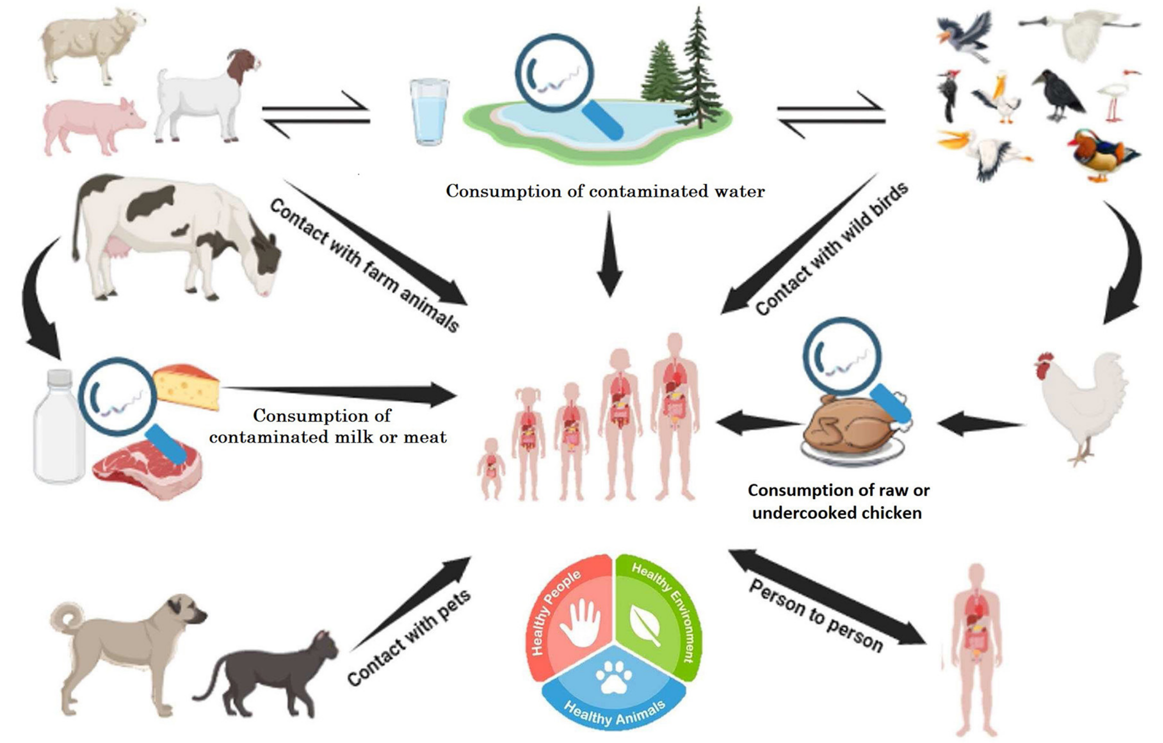

FIGURE 1
Reservoir and transmission of *Campylobacter*. *Campylobacter jejuni* can survive in various animals (poultry, cattle, and wild birds) and water reservoirs. Humans can acquire *C. jejuni* from consuming contaminated meat or meat products [(14); Ali and Alsayeqh].

e.g., antimicrobial effects, antioxidant activities, decreased platelets, detoxification of enzymes for modulation, anticancer properties, and hormonal modulation (25). These effective drug molecules block the replication in maturity of virus in the host body.

It is essential that clinical solutions to prevent diseases should be the last choice. For example, contaminated hands can also transmit the virus to people, fomites and other surfaces, potentially allowing complex additional transmission during any pandemic or epidemic. The elements of safe water, sanitation, and hygiene (WASH) can also limit transmissible disease transmission, including of COVID-19, though reducing respiratory spread is the most important concern (26). Other preventive measures for COVID-19 include isolation, quarantine, physical distancing (including lockdown), and personal protective equipment, including masks (27, 28). Khatib et al. identified through their reviews that the timely assessment and implementation of WASH along with other public health mediates could be essential ensuring acceptable preventive measures.

Environmental protection and remediation are the next concerns after the public safety in One Health mission. Ethylenediaminetetraacetic acid (EDTA) is widely used as an effective chelator for removing hazardous metals from soil (29) and other studies used phytoremediation for metals with or without using EDTA as a phytochelator, or specific types of plants/microbes as phytoremediators (30, 31). They concluded that EDTA could boost the metal uptake capacity. In a separate study, Ejaz et al. identified that Pb and Cd concentrations were significantly higher in shoots of *P. hysterophorus* plants than the roots regardless of EDTA during the treatment processes. However, the presence of EDTA enhanced the accumulation of cations K^+, Na^+, and Ca^{+2} in *P. hysterophorus*. The presence of EDTA increased further accumulation capacity of Pb and Cd on *P. hysterophorus* in soil. Chen et al. (32) demonstrated that phytoextraction of heavy metals from soil was enhanced by the high-biomass plants in presence of concentrated chelate-solubilized materials. These observations verified the importance of natural remediation process and its enhancement in presence of chelator. The plant ecosystem has been contributing significantly to remediation, drug molecule sources, greenhouse gas (GHG) minimization, carbon sequestration, air quality, human health, and others.

Carbon sequestration is the process of capturing and storing atmospheric carbon in the biosphere, mostly determined by the biotic resoluteness of plants including tree diameter, density, and probably stand basal areas (33, 34). Nagendra and Ostrom (35) noticed that the individual tree height is not associated with its diameter, but Feldpausch et al. (36) addressed the correlation between the height-diameter ratio and genetic nature of the species. The intraspecific competition, and abiotic drivers like precipitation, temperature, and soil types also play significant role in the sequestration process (37). Elevation is inversely proportional to the tree height (38); however, in the past the researchers used diameter at breast height (DBH) data of the tree ensuring the progress of and estimating carbon sequestration. Recently, tree height data has been used instead of DBH for more precise estimation of carbon (39). In addition, Ali et al. identified abiotic factors that mainly determined carbon sequestration in forest ecosystems along with the elevation gradients. They used a structural equation modeling approach to test a hypothesized causal relationship among the response variables using data from 200 plots covering sub-tropical thorn forests, sub-tropical broad-leaved forests, moist temperate mix forests, dry temperate conifer forests, and dry temperate *Pinus gerardiana* (*Chilgoza*) forest plots. They also quantified the effects of various forest types on carbon dioxide reduction leading to improved air quality. Furthermore, Potter and Woodall (40) concluded that species richness (SR) is also essential but not the most critical and appropriate metric in biodiversity and carbon sequestration.

One Health is a collaborative, multisectoral, and transdisciplinary approach working at the local, regional, national, and global levels. This editorial article briefly summarized 11 interesting studies on antimicrobial-resistant germs and pathogenicity, vector-borne diseases, contamination of the environment, carbon sequestration, antiviral COVID-19 protein, mobilization of worker behavior, propagation of tick-borne viruses, planetary health education framework, and human-animal interactions. The discussions in this edition categorically addressed how the One Health approach can prevent outbreaks of zoonotic disease, improve food safety and security, improve human and animal health by reducing antimicrobial-resistant infections, secure global health, and climate security, and protect biodiversity. We identified that the implementation of the One Health approach is a critical concern, and the possibility to meet the 17 SDGs by 2030 is questionable. Therefore, it is essential to have a more robust database with proper enforcement and regulatory strategies, organized policymakers, coordination of law makers, non-government organizations and stakeholders, and continuous global monitoring to expedite the implementation processes.

Author contributions

SY: Formal analysis, Validation, Visualization, Writing – review & editing. SA: Formal analysis, Validation, Visualization, Writing – review & editing. DS: Formal analysis, Validation, Writing – review & editing. DPF: Formal analysis, Validation, Visualization, Writing – review & editing. MMTK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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WASH to control COVID-19: A rapid review

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Background: Preventive public health has been suggested as methods for reducing the transmission of COVID-19. Safety and efficacy of one such public health measure: WASH intervention for COVID-19 has not been systematically reviewed. We undertook a rapid review to assess the effect of WASH intervention in reducing the incidence of COVID-19.

Methods: We conducted searches in PubMed, MEDLINE, and EMBASE. We undertook screening of studies in two stages and extracted data and assessed the quality of evidence for the primary outcome using GRADE recommendations.

Main results: We included a total of 13 studies with three studies on COVID-19 and 10 on SARS. The study found that hand washing, sterilization of hands, gargling, cleaning/shower after attending patients of COVID-19, or SARS was protective. Evidence also found that frequent washes can prevent SARS transmission among HCWs. However; one study reported that due to enhanced infection-prevention measures, front-line HCWs are more prone to hand-skin damage. The certainty of the evidence for our primary outcome as per GRADE was very low. We did not find any studies that assessed the effect of WASH on hospitalizations, and mortality due to COVID-19. Also; we did not find any study that compared WASH interventions with any other public health measures.

Conclusions: Current evidence of WASH interventions for COVID-19 is limited as it is largely based on indirect evidence from SARS. Findings from the included studies consistently show that WASH is important in reducing the number of cases during a pandemic. Timely implementation of WASH along with other public health interventions can be vital to ensure the desired success. Further good-quality studies providing direct evidence of the efficacy of WASH on COVID-19 are needed.

KEYWORDS

COVID-19, water sanitation and hygiene (WASH), interventions, SARS, public health, sanitation, hygiene

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Introduction

In the last month of 2019, a novel coronavirus, called SARS-CoV-2 emerged in China and caused an outbreak of coronavirus disease 2019 (COVID-19) (1). By January 30, 2020, the World Health Organization (WHO) declared COVID-19 as a Public Health Emergency of International Concern (2, 3). The rise in the number of cases was contributed by person-to-person transmission in family homes, hospitals, and community and intercity (4–6). As of now, there is no known specific, effective, proven, pharmacological treatment. Slowing down the spread of COVID-19 through public health and social measures currently seem the mainstay of tackling the pandemic (7, 8). However, it would be very difficult to maintain the lockdown of institutions and public places and restrict trade and travel indefinitely.

Preventive public health measures such as isolation of cases, quarantine, hand hygiene practices, masks, physical distancing (including lockdown), quarantine, personal protective equipment (PPEs), and other workplace interventions have been suggested as methods for reducing the transmission of COVID-19 (8–10). WASH is the collective terminology for Water, Sanitation, and Hygiene interventions. As these three words are interdependent, these are bracketed together (5). The facility of safe water, sanitation, and hygiene are vital in safeguarding health epidemics of communicable diseases, including the current COVID-19 pandemic (5).

Evidence in hand shows that SARS-CoV-2 is transmitted *via* respiratory droplets (11). Droplets usually land on surfaces where the virus can remain viable. Thus, the area around an infected COVID-19 patient can act as a source of contact transmission. Once hands come in direct contact with the contaminated surface, the contaminated hands can cause self-inoculation by touching the mucous membranes of the nose, mouth, or eyes (5). The contaminated hands can also transmit the virus to another surface, which further facilitates indirect transmission. WASH intervention including hand hygiene is very important in reducing the chances of this self-contamination (12), subsequent nasal inoculation thereby curtailing the spread of the COVID-19 (13). Though SARS-CoV-2 has not been detected in drinking water, conservative methods of water treatment such as filtration and disinfection can deactivate the SARS-CoV-2 as other types of coronaviruses were found to be inactivated by chlorination and disinfection with ultraviolet light (5, 14).

The safety and efficacy of WASH intervention for COVID-19 have not been systematically reviewed. Therefore, we undertook this rapid review to assess the efficacy of WASH interventions in reducing the incidences of COVID-19. The review also sought to assess the effectiveness of WASH intervention in reducing mortality due to COVID-19 and explore any variations in the effectiveness of WASH in different settings.

Rationale

Evidence in hand shows that SARS-CoV-2 is transmitted *via* respiratory droplets. The contaminated hands can also transmit the virus to another surface, which further facilitates indirect transmission. The area around an infected COVID-19 patient can act as a source of contact transmission. Effectiveness of WASH intervention in reducing mortality due to COVID-19 and explore any variations in the effectiveness of WASH in different settings. SARS-CoV-2 has not been detected in drinking water, conservative methods of water treatment such as filtration and disinfection can deactivate the SARS-CoV-2 as other types of coronaviruses were found to be inactivated by chlorination and disinfection with ultraviolet light. Findings from the included studies consistently show that WASH is important in reducing the number of cases during a pandemic. Timely implementation of WASH along with other public health interventions can be vital to ensure the desired success.

Methods

This rapid review has been prospectively registered in Prospero (Registration Number: CRD42020179663) (15). Though we adhered to PRISMA (16) guidelines throughout this manuscript; we curtailed the systematic review methods and adopted the following shortcuts recommended in methodology to undertake this rapid review:

- We restricted the number of comparisons and outcomes.
- We did not undertake searches of gray literature; or contact experts for on-going studies or any authors for missing data.
- During the screening of studies for eligibility criteria, the second reviewer checked 30% of the excluded records in the first phase and 100% of records in the second phase of screening.

Criteria for considering studies for this review

Pre-specified eligibility criteria were as follows:

Study design

We included a broad range of study designs such as cohort studies, case-control studies, time series, interrupted time series, and mathematical modeling studies. We excluded case reports, case series and case studies in this rapid review.

Population

We included studies reporting the efficacy of WASH interventions (irrespective of study setting) in contacts of suspected or confirmed cases, individuals residing in areas with the rising trend in cases, or individuals traveling from areas where COVID-19 outbreaks were declared. We considered "Outbreaks" as an "occurrence of disease cases in excess of normal expectancy" (17). We included studies irrespective of age, gender, race/ethnicity of individuals, or presence of chronic/comorbid conditions. As a piece of indirect evidence for COVID-19, we also included studies on a similar condition SARS. We excluded studies on individuals with symptoms suggestive of COVID-19 such as Middle-East Respiratory Syndrome (MARS) infections, and studies on asymptomatic individuals with a history of exposure to other organisms causing other respiratory infections.

Intervention

We included studies that assessed the efficacy of different types of WASH (Water, Sanitation, and Hygiene) interventions either as a single measure or in combination with other public health measures like quarantine, personal protective equipment's (PPEs), physical distancing including lockdown, other workplace interventions, training; etc. We defined WASH intervention as per the earlier Cochrane reviews on WASH (18). We included different components and types of WASH interventions irrespective of setting (community or hospital). We excluded studies that have reported the efficacy of WASH interventions in combination with other public health measures related to travel.

Comparator(s)

We include studies that compare:

1. WASH interventions with no WASH interventions.
2. WASH interventions vs. any other public health measures (without WASH interventions) like quarantine of individuals or a community, PPEs, physical distancing including lockdown, other workplace interventions; etc.

Outcome(s)

We reported the following outcomes:

Primary outcome:

1. Number of COVID-19 cases (reported as per clinical or lab diagnosis by the authors of included studies).

Secondary outcomes:

1. Hospitalizations (reported as individuals hospitalized for symptoms suggestive of COVID-19 by the authors of included studies).
2. Mortality due to COVID-19 (reported as deaths due to COVID-19 by the authors of included studies).
3. Adverse events (reported as adverse events by the authors of included studies).

We reported data on time points as reported in studies.

Search methods for identification of studies

An information specialist designed and conducted literature searches systematically, which were verified by a content expert (SZQ) and peer-reviewed independently. The information specialist undertook searches in MEDLINE, CENTRAL, and EMBASE. We also searched for the WHO Global Index Medicus (https://www.who.int/library/about/The_Global_Index_Medicus/en/). The detailed search strategies are presented in Supplementary Table 1. Additionally; we screened the reference lists of the included studies and related systematic reviews for identifying potentially relevant studies. As we are not expecting to find any conference abstracts as the conferences have been postponed/rescheduled because of the COVID-19 pandemic, we did not search for conference abstracts.

Screening and selection of studies

We exported all the records identified through a systematic literature search to the Rayyan web-app (19) and removed the duplicates. We undertook screening of records in two stages. In the first stage, one reviewer with expertise in systematic reviewing (MNK) screened all titles and abstracts for eligibility as per the pre-defined inclusion and exclusion criteria and a second reviewer (DS) checked 30% of the excluded records. One reviewer (MNK) then reviewed full texts of all the records deemed eligible in the first stage of screening and the second reviewer (DS) checked all the excluded records. We resolved disagreements by consensus or by involving a third senior reviewer (AS). We recorded all decisions taken during screening and outlined the list of excluded studies separately. We excluded studies published in languages other than English or Chinese. We included Chinese studies only if abstracts or summaries are available in English.

Data extraction

One reviewer (SZQ) conducted data extraction with a pilot-tested form using Excel and a second reviewer (AG) verified the same. We recorded the following data:

- Study design
- Setting

- Participant characteristics
- Intervention characteristics
- Comparator characteristics
- Outcomes assessed
- Numerical data for outcomes of interest
- For modeling studies, we additionally extracted data for the type of model, and data source.

Risk of bias assessment

One reviewer (MNK) conducted a Risk of Bias (RoB) assessment and a second reviewer (PB) verified the same. The RoB was assessed with "Tool to assess the risk of bias in case-control studies" (20) and "Tool to assess the risk of bias in cohort studies" (21). We resolved discrepancies by discussion and involving a third reviewer (AS). Due to time constraints, we did not contact the authors to seek missing information.

Data synthesis

We had planned to synthesize data by conducting meta-analyze only if participants, interventions, comparisons and outcomes are judged to be sufficiently similar and relevant. However; we found diverse types of participants, interventions, methods of measurement, manner of reporting of outcomes in included studies, and subsequent heterogeneity. Hence; we did not pool the results of the included studies in meta-analysis and rather preferred to present a qualitative description of these studies with supporting tables as narrative synthesis. We had planned to quantify heterogeneity by using I^2 statistics and explore possible causes of heterogeneity among study results by undertaking subgroup analysis for the primary outcome in terms of different age and presence/ absence of chronic or comorbid conditions. We had planned to assess reporting biases by inspecting funnel plots for asymmetry (If more than ten studies included in meta-analysis). However; due to lack of studies, we were not able to do so. To explore the possible influence of covariates, we had planned to undertake subgroup analyses for primary outcome stratified by age, and presence/absence of chronic/comorbid conditions. As we did not undertake any meta-analyses, subgroup analysis was not possible. We had planned to conduct sensitivity analyses by excluding studies rated as "high risk" of bias. As we did not undertake any meta-analyses, sensitivity analyses was not possible.

Assessment of the certainty of the evidence

One reviewer (AG) assessed the certainty of the evidence for the primary outcome using GRADE (Grading Quality of Evidence and Strength of Recommendations) (22) recommendations and presented the results in a summary of findings table (Table 1). GRADE uses four categories to classify the certainty of evidence. A "high" certainty rating of a body of evidence means that we were very confident that the estimated effect lies close to the true effect; "moderate" certainty means we assume the estimated effect is probably close to the true effect; a "low" certainty rating suggests that the estimated effect might substantially differ from the true effect; and "very low" certainty means that the estimated effect is probably markedly different from the true effect. Observational studies start with moderate quality of evidence and are downgraded as per assessments of RoB, indirectness, inconsistency, imprecision, and publication bias.

Current state of knowledge

Results of the search

The PRISMA flow diagram (Figure 1) provides an overview of the study selection process. We identified 689 records from electronic searches and five records from other sources. All 435 records that remained after removal of duplicates were screened initially based on title and abstract during which we excluded 414 records and 21 potentially relevant records were subsequently screened based on full-text. Thirteen records met the eligibility criteria and were included in this review. However; due to diverse types of interventions, methods of measurement, and manner of reporting of outcomes and subsequent heterogeneity; we did not undertake quantitative synthesis. We have recorded the reasons for the exclusion of seemingly related studies in a separate table of excluded studies (Supplementary Table 2).

Included studies

We have presented the characteristics of the studies that met the inclusion criteria in "Characteristics of included studies table" (Table 2) and the results of each study in "Results of included studies table" (Tables 3–5). Our searches identified 13 relevant studies (Figure 1). Of these, three focused on COVID-19 (23–25) and 10 focused on SARS (26–33). All three studies addressing COVID-19 were case-control studies conducted in China (23–25). From the 10 studies focusing on SARS, nine were hospital-based case-control studies from China (26–32), Hong Kong (13, 27, 33), Taiwan (31), and Singapore (34), and one modeling study from Taiwan (35). All the participants in the included studies were HCWs. We did not find any study done in community settings on the general population. All the included studies focus on hygiene either alone or in combination with any other public health measures like quarantine of individuals or a community, PPEs, physical distancing, training, prophylactic medicines, other infection control, or workplace interventions; etc. We did not find any study done to assess the effectiveness of sanitation in controlling the pandemic. We did not find

TABLE 1 Summary of findings for the primary outcome (Number of cases of COVID-19).

WASH intervention in combination with other public health measures compared to no intervention for reducing the number of cases of COVID-19

Patient or population: Reducing the number of cases of COVID-19
Setting: Hospital
Intervention: WASH intervention in combination with other public health measures
Comparison: No intervention

Outcomes	No of participants (studies) follow up	Certainty of the evidence (GRADE)	Relative effect* (95% CI)	Anticipated absolute effects	
				Risk with no intervention	Risk difference with WASH intervention in combination with other public health measures
Number of cases of COVID-19 assessed with: Any criteria for labeling a confirmed case of COVID-19 as reported by the authors	0 cases 72 controls (1 observational study)	⊕◯◯◯ Very low[a,b]	Not estimable	Low	
				0 per 1,000	0 fewer per 1,000 (0 fewer to 0 fewer)

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
CI, Confidence interval.
GRADE Working Group grades of evidence.
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
[a] Downgraded once for study design.
[b] Downgraded twice for imprecision due to sparse data and low participant numbers.

any studies that compared WASH interventions with any other public health measures (without WASH interventions) like quarantine of individuals or a community, PPEs, physical distancing including lockdown, other workplace interventions; etc. We found only studies that reported the adverse events related to WASH and no studies that assessed the effect of WASH on mortality, and hospitalizations.

Effects of interventions

Comparison 1: WASH interventions vs. no WASH interventions

Effectiveness of WASH on the number of COVID-19 cases

We identified one retrospective (24) and one prospective study (25) from China that assessed the efficacy of WASH (handwash) and installation of rapid hand sanitizer stations, respectively, in reducing the cases of COVID-19 in HCWs attending to patients of COVID-19. We did not find any modeling study. We report the evidence narratively. The retrospective study from China (24) did not compare WASH intervention with no WASH intervention but compared optimal handwashing practices with sub-optimal handwashing practices, and qualified handwashing with unqualified handwashing. The study highlights the importance of optimal hand hygiene after coming in contact with COVID-19 patients by demonstrating that optimal handwashing practices in HCWs reduce the risk of developing COVID-19 by 59% ($P = 0.003$) as compared to those with suboptimal handwashing practices (Figure 2) (24). The study also compared qualified handwash with unqualified handwash and demonstrated that qualified handwash reduced the risk of developing COVID-19 by 62% ($P = 0.04$) as compared to unqualified handwash (Figure 3). Another prospective hospital-based study (25) from China was conducted on the efficacy of refined prevention and

FIGURE 1
PRISMA flow diagram for inclusion of studies.

control management strategies including installation of rapid hand sanitizer stations in reducing the risk of COVID-19 cases among HCWs and individuals attending non-isolated areas in general hospitals such as outpatients, emergencies, wards, administrative offices with a high-risk of suspected cases. The study found that though the compliance rate for hand hygiene was only 40.78%, no cases of COVID-19 were found.

Overall, we included 10 studies including nine retrospective studies (13, 26–33), and one modeling study (34) that provided indirect evidence for the effect of WASH interventions in reducing the cases of SARS. The retrospective case-control studies used data from China (29–35), Hong Kong (13, 27, 33), Taiwan (32, 34), and Singapore (30) during or after the SARS outbreak in 2003. The modeling study (36) relied on data from SARS outbreaks in Taiwan.

Evidence on handwash

Six case-control studies and one modeling study assessed handwashing practices (13, 27, 30) and the availability of handwashing facility in hospitals (32–34) as a protective factor in controlling SARS infection in HCWs attending patients during the epidemic.

A study by Lau et al. (27) found that out of 330 individuals, who developed SARS, 61 individuals, washed hands 11 or more times per day, and that out of 660 individuals, who did not develop SARS, 61 individuals washed hands 11 or more times per day. The study concluded that washing one's hands more than 10 times a day is a significant protective factor and along with other public health, measures may have contributed substantially to the control of SARS epidemic in Hong Kong [Matched univariate OR (95% CI): 0.44 (0.31–0.63), $P < 0.005$]. Another study case-control study hospital-based study conducted in five Hong Kong hospitals in 254 participants with exposure to 11 index patients of SARS during patient care (13). Out of 13 HCWs infected with SARS, 10 (77%), HCWs did handwashing and out of 241 patients that did not develop SARS, 227 (94%) HCWs did handwashing [OR (95%CI) = 5 (1–19), $P = 0.022$]. The study found that no staff became infected when they used hand-washing with PPE (13). A similar case-control hospital-based study by Teleman et al. (31) in Singapore undertaken to study the risk and protective factors for nosocomial transmission of SARS in a hospital during SARS outbreak found that out of 36 HCWs that developed SARS, 27 (75%) reported handwashing, while out of 50 HCWs that did not develop SARS, 46 (92%) reported handwashing [OR (95% CI) = 0.06 (0.007–0.5), $P = 0.03$]. The study found that hand washing after attending patients was found to be strongly protective with a 15-fold amelioration of odds (31).

Few studies (33, 35, 36) also assessed the protective effect of the installation of a handwashing facility in controlling SARS infection in HCWs. Yen et al. (36) conducted a Modeling study in 48 hospitals of Taiwan that provided hospitalization for 664 SARS patients. The study was conducted to determine the effectiveness of infection control measures (ICMs) by logistic regression and structural equation modeling (SEM); a quantitative methodology that can test a hypothetical model and validates causal relationships among infective control measures. Sixteen hospitals had episodes of infection of SARS in HCWs. The logistic regression analysis showed that the installation of handwashing stations in emergency departments

TABLE 2 Characteristics of included studies.

Author and country	Study design	Intervention	Outcome
Ran et al. (25) China	Retrospective tertiary hospital-based setting	Qualified v/s unqualified handwashing Optimal v/s suboptimal hand hygiene Co-intervention: and/or PPE	Incident cases of SARS-CoV-2
Chen et al. (27) China	Retrospective hospital based study	Washing hands, nasal cavity and oral cavity (Frequency) Other factors assessed: PPE, training for SARS, ventilation, etc.	Cases of SARS
Lan et al. (24) China	Retrospective study Hospital-based	Handwashing with water and soap Other intervention: Other enhanced infection prevention measures	Adverse events of excessive handwash
Lau et al. (28) Hong Kong	Case-control study	Hand wash (Washed hands 11 or more times per day; Reference = 1–10 times/day) Other measures assessed: face masks, disinfections	Cases of SARS
Liu et al. (29) China	Case control study	Nose wash; Other protective measures: PPE, trainings, prophylactic medicine	Cases of SARS
Pei et al. (30) China	Case control study	Sterilization of hands, untouched hand-washing equipment's, gargling after contact with patients, cleaning oneself thoroughly when off duty were protective	Cases of SARS
Seto et al. (13) Hong Kong	Case-control hospital-based study (n = 5)	Hand-washing other factors assessed: masks, gloves and gown	Cases of SARS
Teleman et al. (30) Singapore	Case-control hospital-based study	Handwashing other protective measures assessed: N95 masks, gloves and gowns	Cases of SARS
Yen et al. (34) Taiwan	Modeling study by Structural Equation Modeling	Handwashing other protective measures assessed: other infection control measures	Cases of SARS
Yen et al. (33) Taiwan	Retrospective Hospital-based study	Traffic control bundle (Triage and diversion of patient before entrance to hospital, delineation of zones of risk, and hand disinfection at checkpoints between zones of risk)	Cases of SARS
Yin et al. (34) China	Case-control hospital-based study (n = 10)	Hand-washing and disinfection, gargle, shower, and changing clothing after work. Other protective measures assessed: PPE, avoidance from eating and drinking in ward, oseltamivir	Cases of SARS
Yu et al. (35) China and Hong Kong	Case-control hospital-based study (86 wards in 21 hospitals in Guangzhou and 38 wards in five hospitals in Hong Kong)	Washing facility Co-intervention: availability of changing facilities, minimum distance between beds of $\leq$1 m, use of an exhaust fan, use of high-flow-rate O_2 mask, performance of resuscitation, staff working while experiencing symptoms, and a workload of <2 patients per one HCW	Incident cases of SARS

was significantly associated with the protection of HCWs from developing SARS [OR (95%CI) = 1.07 (1.02–1.14), $P = 0.012$] (34). The study concluded that hospitals with better infection control measures are less likely to have HCWs acquiring SARS (34). Yen et al. (31) conducted a hospital-based retrospective study in one of the epicenters Taiwan to determine most effective factors in preventing nosocomial infections of HCWs during the 2003 SARS epidemic. Out of 19 hospitals with one or more HCWs with a nosocomial SARS infection, 6 (31.6%) hospitals installed hand-washing stations in emergency departments, and 5(26.3%) around the whole hospital. Out of 31 hospitals with no nosocomial SARS infection among HCWs, 28 (90.3%) hospitals installed hand-washing station in emergency departments ($P <$ 0.001), and 20 (64.5%) hospitals had a set-up of hand-washing facilities around the whole hospital ($P < 0.009$) (31). The study concluded that the installation of a hand-washing station in emergency departments and around the whole hospital was significantly associated with effective prevention of nosocomial SARS infection during the SARS epidemic (31). Yu et al. (33) conducted a case-control hospital-based study (86 wards in 21 hospitals in Guangzhou and 38 wards in five hospitals in Hong Kong). Case wards were hospital wards in which super spreading events of SARS occurred ($\geq$3 new cases of SARS) while control wards were hospital wards in which patients with SARS were admitted, but no subsequent outbreaks occurred. The study found that providing adequate washing or changing

TABLE 3 Results of included studies table (Direct evidences on COVID-19 for primary outcome of Incident cases).

SN	Study ID country	Study design and setting	Outcomes (as reported in studies)	Outcomes (interpretation)	Conclusion
1.	Ran et al. (25) China	Retrospective study Hospital-based setting (Tertiary hospital single-center)	Qualified hand-washing ($n = 22$): infected: $n = 04$ Unqualified hand-washing ($n = 50$): infected: $n = 24$, RR (95% CI) = 2.64 (1.04–6.71), $P \leq 0.05$ Qualified hand-washing v/s unqualified hand-washing: RR (95% CI): 0.38 (0.15–0.96), $P = 0.04$ Optimal hand hygiene before contact patients ($n = 33$): Infected: $n = 06$ Optimal hand hygiene after contact patients ($n = 44$): Infected: $n = 11$ Suboptimal hand hygiene before contact patients ($n = 39$): Infected: $n = 22$, RR (95% CI): 3.10 (1.43–6.73), $P < 0.01$ Suboptimal hand hygiene after contact patients ($n = 28$): Infected: $n = 17$, RR (95% CI): 2.43 (1.34–4.39), $P < 0.01$ Optimal handwash v/s Suboptimal handwash (after contact with patients): RR (95% CI) = 0.41 (0.23–0.74), $P = 0.003$	Optimal handwashing practices in HCWs reduces the risk of developing COVID-19 by 59% ($P = 0.003$) as compared to those with suboptimal handwash	HCWs working with suboptimal hand hygiene after contacting patients had a higher risk of COVID-19.
2.	Xu et al. (26) China	Prospective hospital-based study	Hand washing: 84 out of 206 (40.78%) complied to hand washing Disinfection: disinfection rate of environmental and medical supplies was 100% Cases of COVID-19: Nil	Though the compliance rate for hand hygiene was only 40.78%, no cases of COVID-19 were found	Refined management strategies for the prevention and control of nosocomial infections in HCWs.

facilities for staff was protective [(OR, 0.12; 95% CI, 0.02–0.97), $P = 0.05$] for staff and helped reduce the risk of nosocomial outbreaks. This also submitted that HCWs could act as passive carriers of the SARS coronavirus, which would lead to nosocomial transmission. The study by Chen et al. (26) found that out of 748 frontline HCWs involved in the care of SARS patients in two hospitals in China; 91 HCWs developed SARS. The study also compared the frequency of washing hands, nasal cavity, and oral cavity after caring for SARS patients and found that frequently washes can prevent SARS transmission among HCWs.

Evidence on sterilization of hands

Studies also assessed the efficacy of sterilization of hands (29) and the availability of sterilization set-ups in hospitals (31) in controlling SARS. A case-control study conducted by Pei et al. in China, (29) found that out of 147 HCWs infected with SARS, 11 (7.5%) HCWs sterilized hands by iodine after contact with patients and out of 296 patients those did not develop SARS, 105 (39%) individuals sterilized hands by iodine after contact with patients [OR (95%CI): 0.14 (0.25–0.452), $P = 0.00$] (29). Another retrospective hospital-based study (33) from one of the epicenters of SARS in Taiwan found that out of 19 hospitals with one or more HCWs with a nosocomial SARS infection, 10 (52.6%) hospitals had a disinfectant solution available at the main entrance (of the hospital), and 5 (26.3%) hospitals had a set-up alcohol dispenser at checkpoints for glove-on hand rubbing between zones of risks. Out of 31 hospitals with no nosocomial SARS infection among HCWs, 30 (96.8%) hospitals had a disinfectant solution available at the main entrance (of the hospital) ($P < 0.001$), and had a set-up alcohol dispenser at checkpoints for glove-on hand rubbing between zones of risks ($P < 0.001$) (31). Stepwise logistic regression model of SARS prevention in hospitals of Taiwan found that set-up of alcohol

TABLE 4 Results of included studies table (Indirect evidences on COVID-19 from SARS cases for primary outcome of Incident cases).

SN	Study ID and country	Study design and setting	Outcomes (interpretation)
1.	Chen et al. (27) China	Retrospective hospital-based study	Out of 748 frontline HCWs involved in care of SARS patients in two hospitals in China; 91 HCWs developed SARS.
2.	Lau et al. (28) Hong Kong	Case-control study	Out of 330 individuals, who developed SARS, 61 individuals washed hands 11 or more times per day. Out of 660 individuals, who did not develop SARS, 61 individuals washed hands 11 or more times per day.
3.	Liu et al. (29) China	Case control study	Out of 477 HCWs with SARS, 193 (40.46%) washed nose.
4.	Pei et al. (30) China	Case control study	Out of 147 infected SARS HCWs, 11 (7.5%) HCWs sterilized hands by iodine, 9 (6.9%) did gargling, and 109 (82%) cleaned themselves thoroughly after contact with patients. Out of 296 patients those did not develop SARS, 105 (39%) individuals sterilized hands by iodine, 38 (13.5%) individuals did gargling, and 261 (92.2%) cleaned themselves thoroughly after contact with patients.
5.	Seto et al. (13) Hong Kong	Case-control hospital-based study ($n = 5$)	Out of 13 infected SARS HCWs; 10 (77%) HCWs did handwashing and out of 241 patients that did not develop SARS, 227 (94%) HCWs did handwashing
6.	Teleman et al. (32) Singapore	Case-control hospital-based study	Out of 36 HCWs that developed SARS; 27 (75%) reported handwashing. Out of 50 HCWs that did not develop SARS, 46 (92%) HCWs reported handwashing.
7.	Yen et al. (36) Taiwan	Modeling hospitals ($n = 48$)	Installation of hand washing stations in ED was significantly associated with protection of HCWs from developing SARS
8.	Yen et al. (33) Taiwan	Retrospective Hospital-based study from epicenter of the SARS epidemic	Out of 19 hospitals with one or more HCWs with a nosocomial SARS infection, 6 (31.6%) hospitals installed hand-washing station in EDs, 10 (52.6%) hospitals had disinfectant solution available at hospital entrance, 5 (26.3%) hospitals had a set-up of hand-washing facilities around whole hospital, and 5 (26.3%) hospitals had a set-up alcohol dispensers at checkpoints between zones of risks. Out of 31 hospitals with no nosocomial SARS infection among HCWs, 28 (90.3%) hospitals installed hand-washing station in EDs, 30 (96.8%) hospitals installed hand-washing station in EDs, 20 (64.5%) hospitals had a set-up of hand-washing facilities around whole hospital, and 30 (96.8%) hospitals had a set-up alcohol dispensers at checkpoints for glove-on hand rubbing between zones of risks.

TABLE 5 Results of included studies table (Direct evidences on COVID-19 for secondary outcome of adverse events).

SN	Study ID country	Study design and setting	Outcomes (as reported in studies)	Conclusion
1.	Ran et al. (23–25) China	Retrospective study Hospital-based	321 out of 526 HCWs washed their hands >10 times per day reported more hand-skin damage [OR (95%CI) $= 2.17$ (1.38–3.43), $P = 0.01$] 113 out of 526 HCWs washed their hands ≤ 10 times per day Adverse events of excessive handwash (>10 times per day): itching 276 (52.5%) Out of 542 front-line HCWs for COVID-19; 526 (97%) reported hand-skin damage by enhanced infection-prevention measures.	Prevalence of skin damage of first-line HCWs managing COVID-19 is high. Longer exposure time was a significant risk factor for

HCWs, health care workers; OR, odds ratio; CI, confidence interval; MLR, multivariate logistic regression; ED, emergency departments.

FIGURE 2
Forest plot showing effect of optimal handwash as compared to suboptimal handwash on the number of COVID-19 cases.

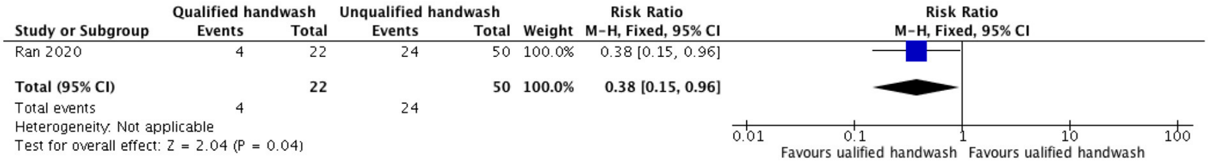

FIGURE 3
Forest plot showing effect of qualified handwash as compared to unqualified handwash on the number of COVID-19 cases.

dispensers at the checkpoint for glove-on hand rubbing between zones of risk was effective [OR (95%CI) 0.043 (0.003–0.627); $P = 0.021$] (31).

Evidence on nose wash

One study by Liu et al. (28) conducted a case-control study in China on 477 HCWs (representing 90% exposed to SARS patients) from Armed Forces Hospital with a nosocomial outbreak of SARS. The study found that out of 477 HCWs with SARS, 193 (40.46%) washed the nose. Reduction in ORs was achieved by washing the nose after attending to patients ($P = 0.0002$). The study thus concluded that nose washing was protective against infection. Also; significant correlations were found between performing nose wash and taking training (Correlation coefficient: 0.144, $P = 0.004$).

Evidence on gargling

One case-control study by Pei et al. (29) found that out of 147 HCWs infected with SARS, 9 (6.9%) HCWs did gargling and out of 296 patients did not develop SARS, 38 (13.5%) individuals did gargling after contact with patients [OR (95%CI): 0.474 (0.22–1.01), $P = 0.049$].

Evidence on cleaning/shower after duty

Two studies (29, 32) assessed the protective effects of cleaning after attending SARS patients. Case-control study by Pei et al. (29) found that out of 147 HCWs infected with SARS, 109 (82%) HCWs cleaned thoroughly after contact with patients and out of 296 patients that did not develop SARS, 261 (92.2%) cleaned thoroughly after contact with patients [OR (95%CI): 0.38 (0.20–0.71), $P = 0.002$] (29).

The study concluded that nosocomial infection of SARS can be avoided by adopting comprehensive protection measures (29). Another hospital-based case-control study by Yin et al. (32) in ten hospitals of China on HCWs involved in direct first aid for severe SARS patients found a dose-response relationship in taking shower and changing clothes after work ($P < 0.01$). The study also found that if more protective measures are used, the protective effect is higher ($P < 0.001$), and that the protective effect was 100% of all interventions were used at the same time.

Evidence on hospitalizations

None of the included studies reported data on hospitalizations of patients for symptoms suggestive of COVID-19 or SARS after WASH interventions as compared with no WASH interventions.

Evidence on mortality due to COVID-19 or SARS

None of the included studies reported data on mortality due to COVID-19 or SARS after WASH interventions as compared with no WASH interventions.

Evidence on adverse events due to WASH intervention

Only one retrospective hospital-based study (23) provided direct evidence of adverse events of excessive handwash caused by enhanced infection prevention measures in front-line HCWs during the COVID-19 pandemic. The study found that out of 542 front-line HCWs for COVID-19; 526 (97%) reported hand-skin damage due to enhanced infection-prevention measures. The study also demonstrated that longer exposure time was a significant risk factor as HCWs that washed their hands more than 10 times per day reported more hand-skin damage [OR (95%CI) = 2.17 (1.38–3.43), $P = 0.01$].

Comparison 2: WASH interventions vs. any other public health measures (without WASH interventions)

None of the included studies reported data on the effectiveness of WASH vs. any other public health measures (without WASH interventions) such as quarantine of individuals or a community, PPEs, physical distancing including lockdown, other workplace interventions; etc. on the number of COVID-19 or SARS cases, hospitalizations, mortality or any adverse events related to WASH.

GRADE assessment

We rated the certainty of evidence as very low for primary outcomes (number of cases). We downgraded one level due to high risk of bias in study design and twice for imprecision due to sparse data and low participant numbers (Table 4).

Discussion

Summary of main findings

To the best of our knowledge, this is the first rapid review of the effectiveness of WASH intervention to control COVID-19. The evidence base is limited because of the very direct few evidence on COVID-19. The other ten included studies are on SARS and contribute only indirect evidence. One study (25) reported the benefit of refined management strategies including hand hygiene and another study (24) reported the benefit of qualified and optimal hand hygiene practices in reducing the risk of COVID-19 among HCWs after coming in contact with infected patients. Other indirect evidence from previous SARS outbreak also suggests the benefit of hand hygiene, nose wash, gargling, shower and installation of handwash station, hand sanitizer station or shower facility in hospitals to avert transmission of SARS in HCWs. However, this evidence is based on the SARS outbreak, and generalizability to COVID-19 is very limited. In general, the combination of any of the WASH interventions with other prevention and control measures such as PPE, isolation, training, prophylactic medicines, proper ventilation, and other infection control measures had a greater effect on reducing the number of cases than individual measures.

Overall completeness and applicability of the evidence

Person-to-person transmission of nCoV-2 has occurred in families, homes, colonies, hospitals, and between cities, states, countries, and continents. Many HCWs and other contacts of infected patients have been affected with COVID-19 after coming in contact with the infected patients. This has led to concern among workers and other contacts who are at risk of being infected while performing their duties. Looking at the current pattern of spread; public health measures and alternative medication are pressing management strategies against the COVID-19 pandemic (35). This pandemic has drawn the attention toward the importance of public health measures, such as personal hygiene, personal protective equipment, isolation of cases, quarantine, physical distancing, other workplace interventions; etc. Hygiene and Public Health are vital to a larger population (36).

SARS was the first pandemic of the twenty-first century which was ultimately brought under control through public health measures such as hygiene practices (e.g., frequent hand washing, face mask, and disinfecting living quarters), travel restraints, and quarantine (37). Infections among HCWs have been a common feature of SARS since it surfaced. It was observed that the majority of SARS cases occurred in settings where infection control measures had not been installed or established or had been installed or established but were not adhered to. CDC had recommended infection control measures such as careful hand hygiene, use of negative-pressure isolation rooms, N95 masks, gloves, gowns, and eye protection (38).

The generation of viral aerosol by a COVID-19 patient suggests a possibility of respiratory droplets transmission. The touchable surfaces in contact with infected patients can be contaminated by infected patients either through respiratory secretion or through hands. This underlines the necessity of suitable respiratory protection and also stringent surface hygiene practices. Conserving a hygienic environment can be one of the valuable public health measures to tackle such infectious diseases. Hand hygiene is a very simple and cost-effective public health infection control measure to prevent the spread of the infectious agent. Quarantine, hygiene measures, and protective equipment were the principal preventive measures that were found to be effective in limiting the spread of SARS in many countries (39). Suboptimal hand hygiene after contacting patients were linked to COVID-19 (24). The society also needs to be educated, supported, and prepared with the skills to foster better health and hygiene.

Limitations in the body of evidence

We did not find any study that directly evaluated the effects of WASH alone or in combination with other measures to control COVID-19. Lack of data may be explained by the fact that the pandemic is still in progress, and such studies may be in progress. The majority of best available evidence in this review is from indirect evidence from nine case-control and one modeling study on SARS. Hence, the applicability and generalizability of evidence from studies on SARS is possibly limited because of different trajectories due to variations in transmission dynamics. Nevertheless, they back the findings for COVID-19.

Potential biases in the review process

Due to the paucity of time, we conducted this rapid review and curtailed the steps adopted in systematic review methods and implemented some shortcuts in our methodology. We did not undertake searches of gray literature; or contacted experts for on-going studies or any authors for missing data. Moreover, we limited publications to the English language. As this pandemic is spreading rapidly, especially in countries like Italy and Spain, there remains a possibility that we may have missed studies conducted recently in these countries. During the screening of studies for eligibility criteria, the second reviewer checked 30% of the excluded records in the first phase and 100% of records in the second phase of screening. One reviewer conducted the 'Risk of bias' assessment, a second review author checked the acceptability and accuracy. This might have introduced some bias to this rapid review. However, in spite of these limitations, we are confident that none of these procedural curbs would have altered the general conclusions of this rapid review.

Summary and perspective

Implications for practice

Current evidence of WASH interventions for COVID-19 is limited due to lack of primary data on novel coronavirus infection and as is largely based on indirect evidence from SARS. Findings from the included studies consistently show that WASH is important in reducing the number of cases during the pandemic. Timely implementation of WASH along with other public health interventions can be vital to ensure better results. The policymakers will have to constantly supervise the pandemic situation and based upon the effect of the implemented public health measures in studies; suggest the best combination of public health interventions.

Implications for research

Although the studies point toward the effectiveness of WASH interventions to control a pandemic, further good quality studies providing suitable, reliable and affordable direct evidence of the efficacy of WASH alone or in combination with other public health measures to control the cases and mortality due to COVID-19 as well as to control such public health emergencies are needed. Studies are also needed on the efficacy of sanitation (a component of WASH) alone or in combination with other public health measures to reduce the cases, hospitalizations, and mortality due to COVID-19 are also needed.

Author contributions

MK contributed to the overall design, coordination, screening of studies against the eligibility criteria, assessment of RoB, and drafted the manuscript in consultation with the co-authors. AS contributed to the design, resolved discrepancy in screening of studies, and provided inputs on all drafts and the final version. GM overall manuscript drafting and reviewing. SQ contributed to the design, provided inputs related to statistics, and provided inputs on all drafts and the final version. SG contributed to the design and provided inputs on all drafts and the final version. DS and SS contributed to the design, screening of studies against the eligibility criteria, and provided inputs on all drafts and the final version. AG contributed to the design, co-ordination, assessed the certainty of the evidence, and provided inputs on all drafts and the final version. PB contributed to the design, verified the assessment of RoB, and provided inputs on all drafts and the final version. SS contributed reviewing the manuscript and provided inputs on all drafts and the final version. QZ contributed to the design, co-ordination, verified systematic literature searches, and drafted the manuscript in consultation with the MK, AS, GM, SQ, SG, DS, AG, PB, and SS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Antiviral COVID-19 protein and molecular docking: *In silico* characterization of various antiviral compounds extracted from *Arisaema jacquemontii* Blume

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Arisaema jacquemontii Blume is a highly medicinal and poisonous plant belong to the family Araceae. It is used to treat several deadly diseases, including viral infections. It has antioxidant, anti-cancerous, antimalarial, anti-vermicidal, and antiviral activities. Therefore, five parts of the *Arisaema jacquemontii* Blume plant, such as leaf, seed, stem, pulp, and rhizome extract, were evaluated for metabolic and *in silico* characterization of probable compounds using gas chromatography-mass spectrometry (GC-MS) analysis. A total of 22 compounds were isolated from the methanolic extracts of *A. jacquemontii* Blume. A selected antiviral COVID-19 protein i.e., protease (6LU7) was docked against the obtained compounds. Different affinities were obtained through various compounds. The best results were shown by three different compounds identified in the rhizome. The maximum binding affinity of these compounds is 8.1 kJ/mol. Molecular docking (MD) indicate that these molecules have the highest binding energies and hydrogen bonding interactions. The binding mode of interaction was discovered to be reasonably effective for counteracting the SARS virus COVID-19. The findings of this study could be extremely useful in the development of more phytochemical-based COVID-19 therapeutics.

KEYWORDS

antioxidant, *in silico*, docking, interaction, phytochemical, *A. jacquemontii* Blume, Cobra lily, Anti-COVID protein

Introduction

Phytochemistry outlines the morphology of a large number of secondary metabolites. Many active ingredients have been discovered and isolated from different parts of plant species, including carotenoids, sterols, aliphatic compounds, monoterpenes and sesquiterpenes, triterpenoids, and other miscellaneous chemicals (1, 2). More than 4,000 phytochemicals have been described and categorized (3). The plant-originated chemicals contain secondary metabolites. These are secreted against when different calamities are faced (4), for example, antimicrobial effects, antioxidant activities, a decrease in platelets, detoxification of enzymes for modulation, anti-cancerous properties, and hormonal modulation (5, 6). Medicinal plants play an essential role in the provision of various phytochemicals. The era of modern research started in the early 19th century after herbal preparation. The first isolated active alkaloids were morphine, quinine, and strychnine (7). The primary emphasis was made on plant-derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation (8–12).

Metabolomics is the large-scale study of small molecules known as metabolites within cells, bio-fluids, tissues, or organisms. These molecules and their interactions within the biological system are collectively known as metabolomes (13–16). These biomolecules provide biologically relevant endpoints of metabolic processes focusing on the products of interactions between gene expression, protein expression, and cellular environment (17, 18). Metabolomics is the most modern omics technology in which the patterns are identified to function and balance the metabolomic changes observed in their pathways (19). Metabolomics is the most comprehensive analysis used to study the diverse metabolites throughout the globe. These are present in different cells of the organisms, vastly expanded to fingerprinting and profiling metabolites through their selected and recognized markers (20). Metabolomics is the most fundamental classical and biological technique that quickly reflects the genotype (21–26). Metabolomics provides information about the qualitative and quantitative data and exhibits a compound's point and specific time. There are more than 200,000 metabolites extracted from plants (27). In the post-genomic era, 30–50% of the sequenced data was not interpreted correctly. Metabolomics is one of the most crucial areas contributing much toward the prokaryotic genome elucidation (28, 29). While out of 1–5 gene functions, only one gene's functions seem to be understood and illustrated in the bacterial genome, such as *E. coli* has an unknown sequence (30). These sequences helped to find out the functional and non-functional genes.

The most common technique for measuring metabolism is mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy (31, 32). These two approaches easily observe different compounds from the plant at various stages. Gas chromatography-mass spectrometry is the oldest technique and the most sensitive, robotic, intensive, and developed technique mainly used to identify the primary metabolic compounds in mass spectrometry, such as carbohydrates, lipids, amino acids, fatty acids, and organic acids (33). In addition, molecular docking is a molecular modeling approach in which we evaluate the interaction of two molecules and anticipate how a protein interacts with a tiny molecule called a ligand, and the modeling technique is called molecular docking. In this developing world of complexity, the area of rational drug design has played a critical role in the development of novel medications (34). At the same time, selecting the biomolecule of interest is the most essential and critical step in rational drug design (35).

A. jacquemontii Blume is a popular Himalayan medicinal plant used to treat a variety of infections in traditional medicine (36–43). It is used as a foodstuff, an antidote for snakebite, and a therapy for respiratory infections, eczema, and rashes (44–49). It has anti-proliferative, anticonvulsant, anti-insect, and antiviral properties (50). It was hypothesized that the *A. jacquemontii* Blume plant possesses specific compounds that can be used as an antiviral. Therefore, this study was carried out to isolate and identify compounds from *A. jaquemontii* Blume with antiviral activity and combating ability against COVID-19 protein (6LU7). It will also contribute to the sustainable development goals by maintaining life on land and supporting the consumption and production of *A. jacquemontii* Blume, which will benefit the community medicinally and increase economic growth by reducing poverty.

Materials and methods

Collection and processing of the plant sample

The plant samples were collected from the Western Himalaya Mountains at an elevation of 4,500 m (51–53). All plant parts, such as rhizome stem and leaves, were washed properly with distilled water, while in the case of seed, the pulp was separated and samples were kept at room temperature (25°C) for proper drying. Some samples were frozen at −80°C for further use.

Extraction of compounds

The *A. jacquemontii* Blume samples were weighed and shade dried for 20 days. After drying, different parts of *A. jacquemontii* Blume, such as rhizome, seed, pulp, leaves, and stem, were thoroughly dried and then ground with the help of a grinder into powder form. The dried samples were weighed,

TABLE 1 GC-MS analysis and characterization of different compounds in various parts of *A. jacquemontii* Blume.

Sr. No.	Parts of plant	Name of compound	Molecular formula	Mass to charge ratio	Retention time (min)
1.	Leave	2-Fluoro-6- (trifluoromethyl)- acetophenone	$C_9H_6F_4O$	191	5.869 min
2.	Pulp	Triallylmethylsilane	$C_{10}H_{18}Si$	97	3.23 min
		Propanenitrile, 3- (methylthio)	C_4H_7NS	61	2.587 min
		Octane, 1-(propylthio)-	$C_{11}H_{24}S$	57	5.402 min
3.	Seed	2, 5- dimethyl-3- isopropylpyrazine	$C_9H_{14}N_2$	135	3.676 min
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	191	5.860 min
		Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	74	10.152 min
4.	Stem	Ortho-Methoxyacetophenone	$C_9H_{10}O_2$	135	3.669 min
		4'-Diethylaminoacetanilide	$C_{12}H_{18}N_2O$	191	5.860 min
		Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	74	10.152 min
		2,5-Dimethylcyclohexanol	$C_8H_{16}O$	57	18.836 min
5.	Rhizome	Beta, - methyl xyloside	$C_6H_{12}O_5$	60	2.531 min
		Nonanoic Acid	$C_9H_{18}O_2$	60	3.343 min
		3- decanol	$C_{10}H_{14}O$	59	5.102 min
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	191	5.860 min
		Benzene, 2-methyl-1, 3, 5- trimethyl	$C_{10}H_{13}O_3$	135	3.674 min
		Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	55	6.196 min
		Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	74	10.154 min
		n-Hexadecanoic acid	$C_{16}H_{32}O_2$	55	10.549 min
		Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	74	12.068 min
		p-Hexyloxy nitro benzene	$C_{12}H_{17}NO_3$	55	14.131 min
		Gamma-Sitosterol	$C_{29}H_{50}O$	57	27.751 min

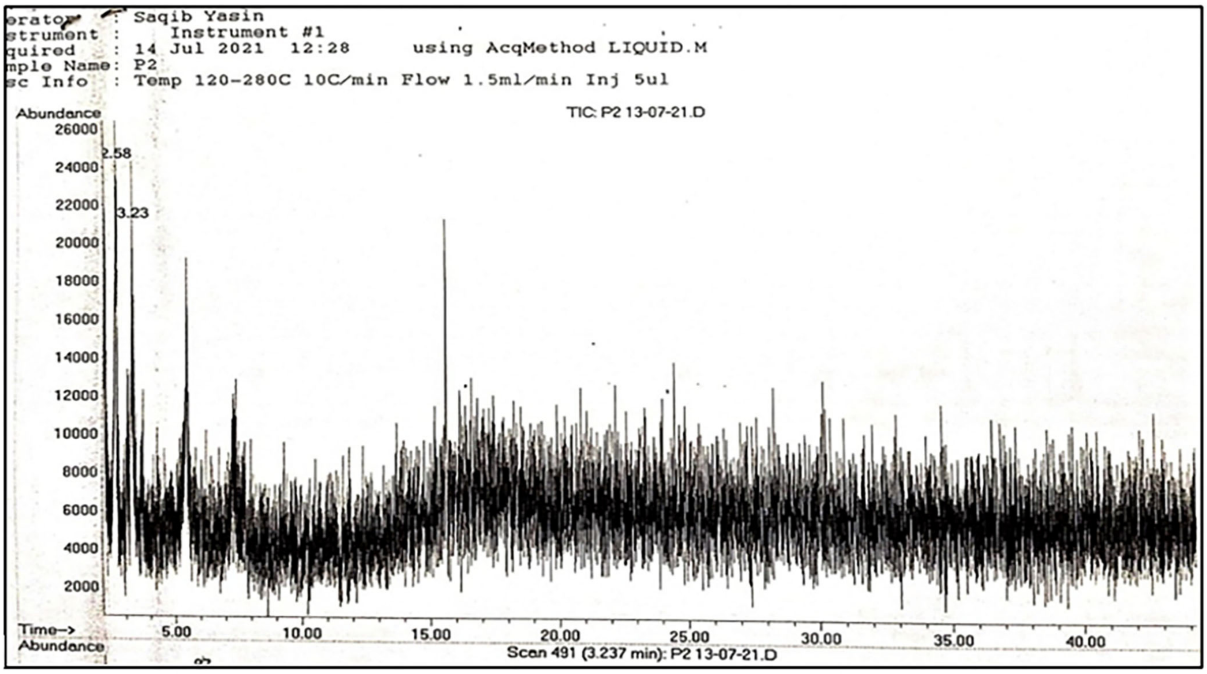
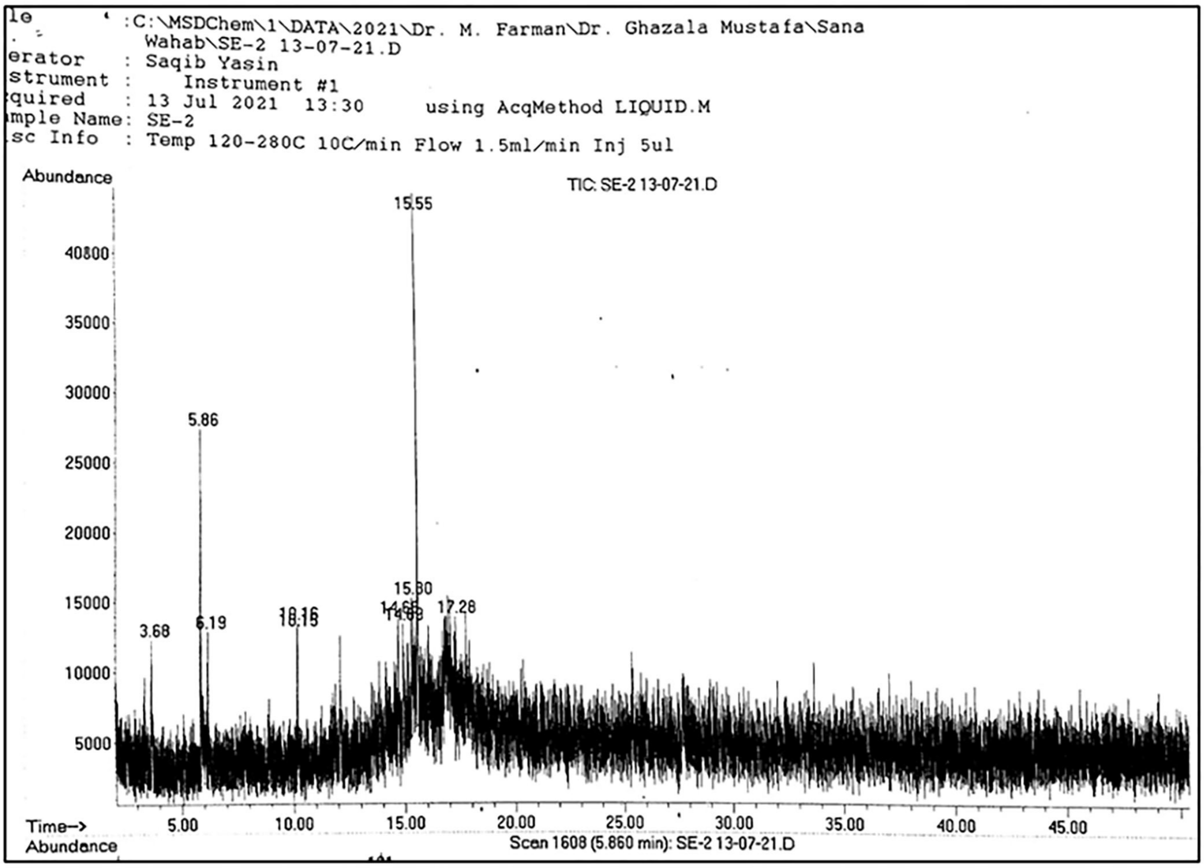


FIGURE 1
Total spectrum obtained through GC-MS of leaf sample.



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mple Name: SE-2
sc Info   : Temp 120-280C 10C/min Flow 1.5ml/min Inj 5ul
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FIGURE 2
Spectrum obtained through GC-MS of pulp samples.

mixed with ethanol at 1 g:10 ml, added methanol, and kept in an electric shaker at 200 rpm (round per minute) for 48 h. After two successive days of shaking, the solution was filtered with the help of Whatmann's No. 1 filter paper. The obtained filtrate from the solution was poured into the Petri plate and placed at room temperature for 1 day to evaporate methanol or chloroform (54). A thin extract layer was obtained in the Petri plate collected in the Eppendorf for future use. After drying and measuring, layer extractions were collected through a spatula in the Eppendorf. This procedure was repeated three times, and the maximum extract was collected for experimentation. The extract was preserved in Eppendorf and used for further research purposes.

Phytochemical screening

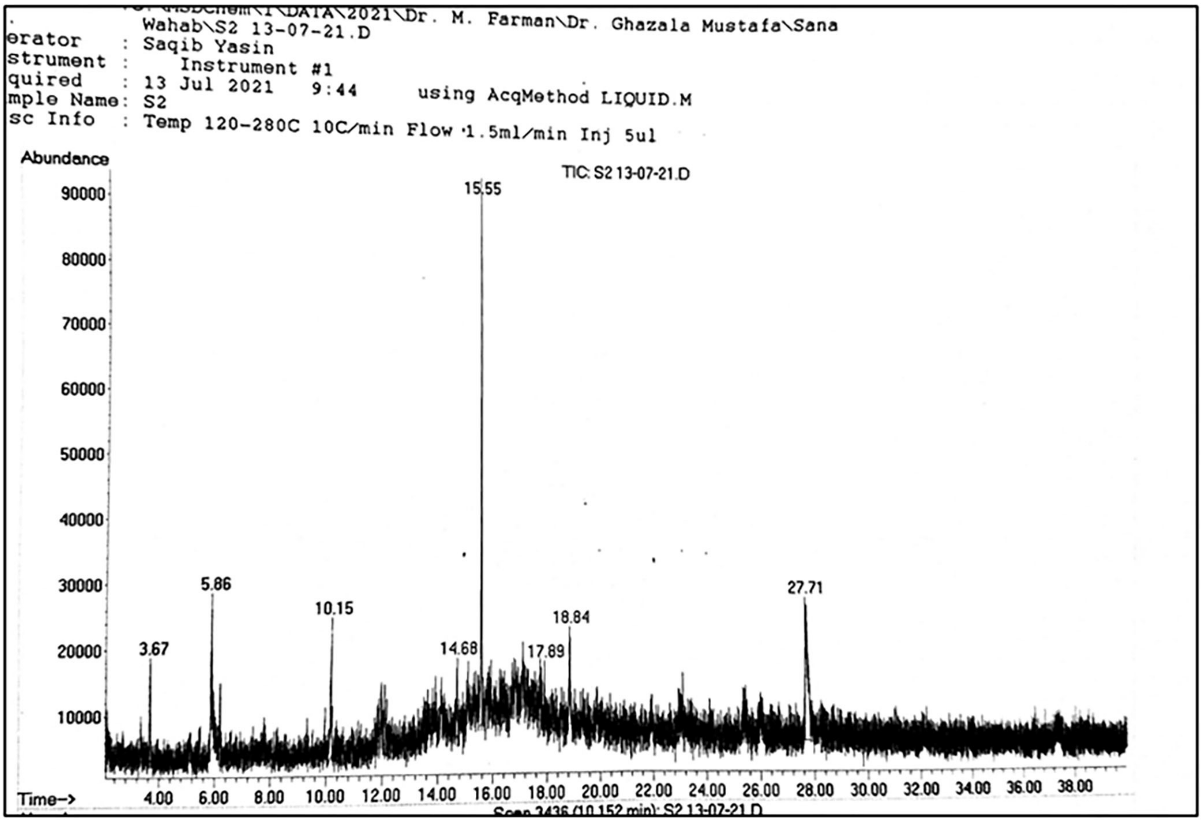
The extract prepared from different parts of the plant species was subjected to standard phytochemical analysis so that various chemical compounds such as alkaloids, flavonoids, tannins, and saponins could be detected (54).

Identification of bioactive compounds in the extract

Methanol and chloroform extracts of different parts of the *A. jacquemontii* Blume were carried out for chromatographic analysis to confirm the presence of phytoconstituents in their active solvent extracts.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS of the plant samples was performed using a PerkinElmer GC Clarus 500 system and chromatograph coupled to a mass spectrometer equipped with an Elite-I, fused silica capillary column (30 mm 90.25 mm ID 91 IMdf, consisting



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strument    :    Instrument #1
quired      : 13 Jul 2021    9:44      using AcqMethod LIQUID.M
mple Name: S2
sc Info     : Temp 120-280C 10C/min Flow 1.5ml/min Inj 5ul
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FIGURE 3
GC-MS spectrum of different compound extracted from seed sample.

of 100% dimethyl polysiloxane). The sample was dissolved in n-hexane and GC-MS identification was operated in electron impact mode with an ionization energy of 70 eV. The National Institute of Standard and Technology (NIST) library database was used for the interpretation of the compounds detected on a mass spectrum (GC-MS) through which the weighed, molecular formula and structural formula of the samples were determined (55).

Molecular docking

Molecular docking is a type of bioinformatics modeling where interactions of two or more molecules give us a stable product. Phytocompounds separate from the highly medicinal plant *A. jacquemontii* Blume using GC-MS analyses, docked against the targeted coronavirus protein to detect the compounds binding with the active site of the targeted *6LU7* protein at chain C (56). This protein is an essential enzyme in the Coronavirus and plays a crucial role in mediating viral replication in the transcription, making it an attractive drug target against SARS-COVID-2. This chain is considered the binding site on the protein for ligand (57). Ligand structures in SDF format were downloaded from PubChem and converted into PDB format through PY mole software. The protein was downloaded directly in PDB format. This format was used to carry out ligand–protein interactions in AutoDock 4.2.6. The protein was prepared for docking by removing water, adding hydrogen, adding kollarman charges, and computing atomic solvation parameters. The protein–ligand complex was designed by creating a grid box in AutoDock 4.2.6 in which all the x, y, and z dimensions are 60, 60, and 60, respectively, with a spacing of 0.5 Å. We have used blind docking to cover the overall protein and grid box. Results in the form of a summary were obtained in the grid.txt file in the respective folder. In this way, protein–ligand interactions were further analyzed by command prompt software to check the affinity of their bonding by running a protein–ligand summary file (58). The LIG PLOT software was used to analyze that protein–ligand complex in three-dimensional structure, their number of hydrogen bonds, the name of that hydrogen bond, and affinity.

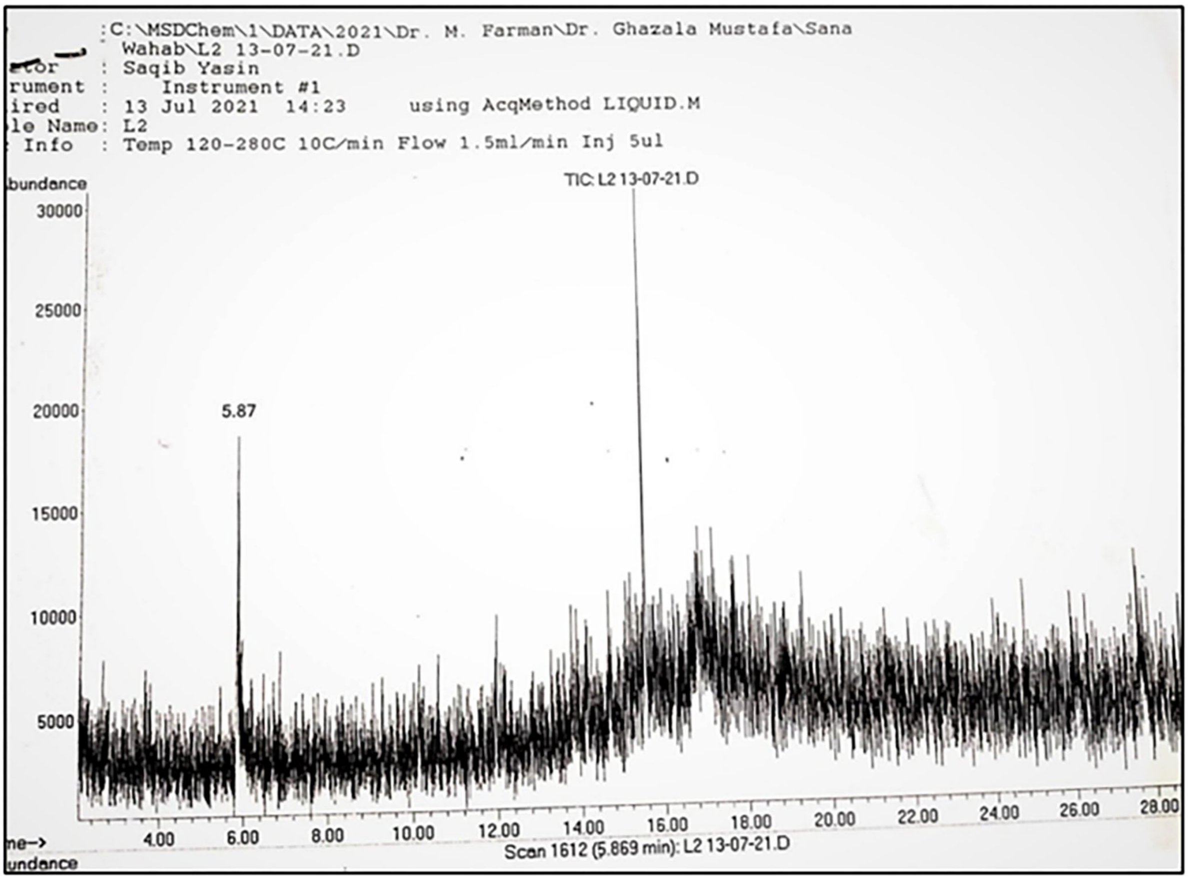

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FIGURE 4
Main spectrum obtained through GC-MS of seed sample.

Results

GC-MS analysis and their characterization

A total of 22 compounds were recorded from *A. jacquemontii* Blume through GC-MS (Table 1). Out of which a leaves extract contains one compound, i.e., 2-fluoro-6-(trifluoromethyl)-acetophenone, pulp extract contains three compounds like triallymethylsilane, propane-nitrile, 3-(methylthio), octane, 1-(propylthio) and seed extract possess three compounds such as 2, 5-dimethyl-3-isopropylpyrazine, phenol, 2, 5-bis (1,1-dimethyl ethyl)-pentadecanoic acid methyl ester (Figures 1–5).

The same number of compounds such as ortho-methoxyacetophenone, 4'-diethylaminoacetanilide, nonanoic acid, methyl ester, and 2, 5-dimethylcyclohexanol were also extracted from the stem. A total of 11 compounds such as beta, methyl xyloside, nonanoic acid, 3-decanol, phenol, 2, 5-bis (1,1-dimethyl ethyl)-, benzene, 2-methyl-1, 3, 5-trimethyl, nonanedioic acid, dimethyl ester, tridecanoic acid, methyl ester, n-hexadecanoic acid, octadecanoic acid, methyl ester, p-hexyloxy nitrobenzene, and gamma-sitosterol were observed in the rhizome of *A. jacquemontii* Blume (Figure 3). Different peaks along with their retention time were observed along with the given names of the compounds (Table 1). According to the literature, only five of these 22 compounds are known for their antiviral activities.

Molecular docking and COVID-19

The GC-MS extract of different parts of *A. jacquemontii* Blume was AutoDocked against antiviral protein *6LU7*. Among all, compounds obtained from rhizome extract yield the highest binding interaction affinity. Ligand–protein interaction was stronger in those compounds (Table 2). The *6LU7* consists of 3C-like protease (3CLpro) and spike protein, a striking goal for medical production in COVID-19. Of all these three chains, the C chain is the binding site for the ligand on protein. This protein is further used in binding interaction with the ligand through

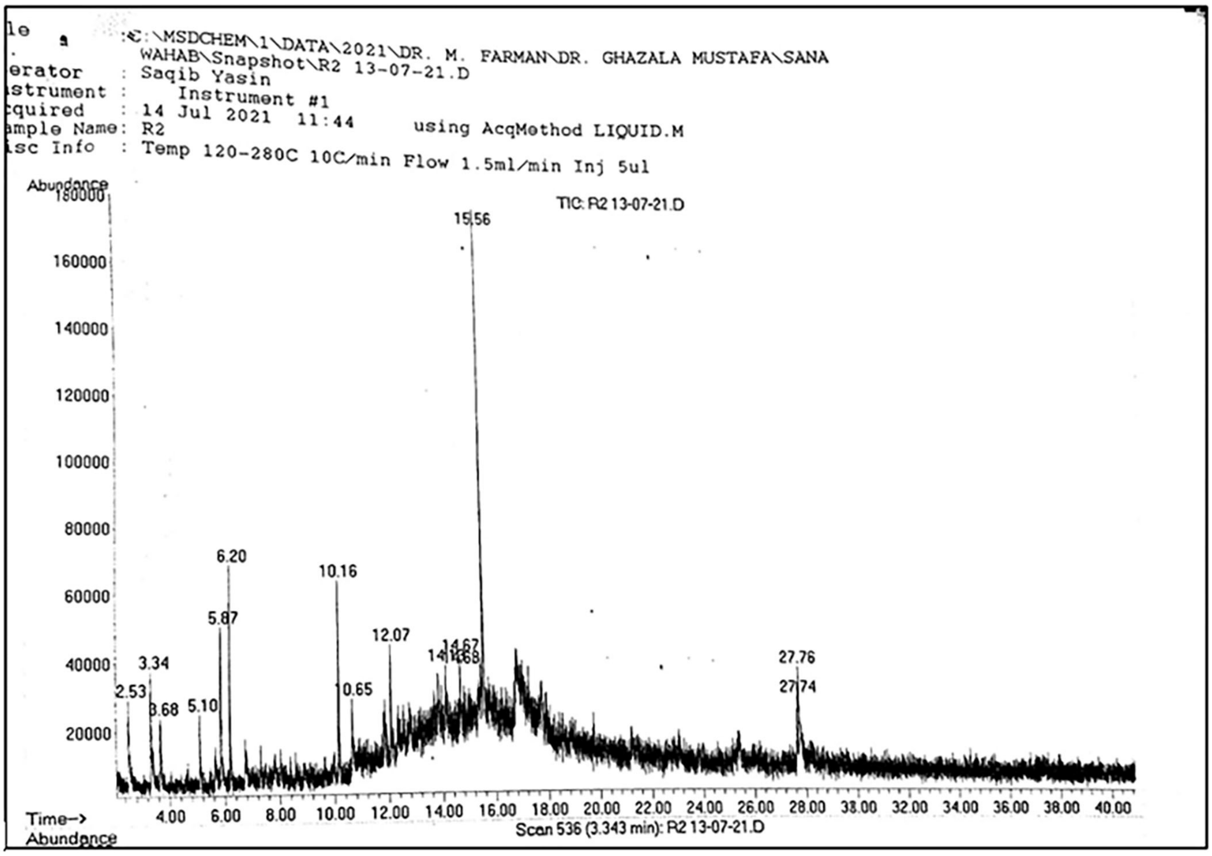


FIGURE 5
Main spectrum showing different peaks observed in Rhizome sample.

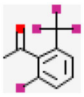
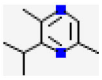
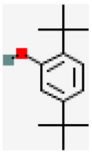
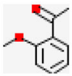
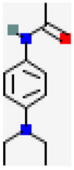
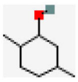
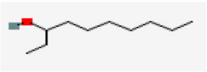
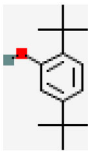
which binding affinity can be analyzed in Auto Dock vina (Figure 6).

The recorded 22 compounds have different binding affinities for the selected protein. These compounds were considered as ligands against interaction with proteins in molecular docking. All the ligands, their structural formula, and their CID number obtained through PubChem and binding affinity calculation are explained in Table 2. Three-dimensional visualization of this entire compound's structure illustrates the number of hydrogen bonds, the name of the hydrogen bond, and hydrophobic interactions with other amino acids. The binding affinities of all the compounds are 2-fluoro-6-(trifluoromethyl)– acetophenone has −7.4 kJ/mol affinity value having only one (1) hydrogen bond O-NH2 having a distance of 2.89 Å and hydrophobic interactions were Phe 219(A), Trp 218(A), Leu 271(A), Gly 275(A), Glu 270(A), and Asn 277(A). Propanenitrile, 3-(methylthio) has −3.4 kJ/mol ligand–protein affinity having two hydrogen bonds N-N, N-OG1 with bond distance 3.20 Å and 2.96 Å, respectively, with hydrophobic interactions Asn 151 (A), Phe 294 (A), Thr 292 (A), and Gln 110 (A). Octane, 1-(propylthio) has a bonding affinity of −5.1 kJ/mol with no hydrogen bond present in it, having hydrophobic interactions as His 246 (A), Val 202 (A), Gln 110(A), Pro 293 (A), Ile 249 (A), and Phe 294 (A), and in the same way, no hydrogen bond was present in 2, 5-dimethyl-3-isopropyl-pyrazine and seven different types of hydrophobic interactions were observed. In the same way, all the compounds showed different numbers and types of hydrogen bonds to varying distances, with quite different binding affinities. The highest binding affinity was −8.1 kJ/mol that was demonstrated in two compounds of rhizome such as p-Hexyloxy nitrobenzene and Gamma-Sitosterol, while no hydrogen bond was present in the first compound and the second one had two hydrogen bonds that were O-O and O-O, with bond distances of 3.03 Å and 3.09 Å, respectively (Supplementary Table 1, Table 3).

Discussion

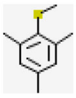
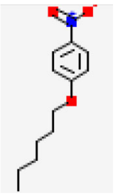
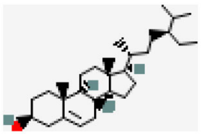
The *Arisaema* genus has been previously analyzed to have antibacterial, antioxidant, and cancer-fighting properties. It has a physiological effect on the human respiratory syncytial virus. The Herpes simplex virus was also examined in the

TABLE 2 Docking results of different parts of *A. jacquemontii* Blume along with their binding affinity.

Sr. No.	Parts of plant	Name of compound	CID number	Structural formula	Affinity (kJ/mole)
1.	Leave	2-Flouoro-6- (trifluoromethyl)- acetophenone	519414		−7.4
2.	Pulp	Trimethylsilane	70435		n.d
		Propanenitrile, 3- (methylthio)	548386		−3.4
		Octane, 1-(propylthio)-	520831		−5.1
3.	Seed	2, 5- dimethyl-3- isopropylpyrazine	518790		−5.3
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	79983		−7.3
		Pentadecanoic acid, methyl ester	23518		−7.2
4.	Stem	Ortho-Methoxyacetophenone	68481		−5.8
		4’-Diethylaminoacetanilide	21400		−6.4
		Nonanoic acid, methyl ester	15606		−5.4
		2,5-Dimethylcyclohexanol	97959		−5.4
5.	Rhizome	Beta, - methyl xyloside	129633168		−6.3
		Nonanoic Acid	8158		−5.3
		3- decanol	519158		−5.8
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	79983		−7.3

(Continued)

TABLE 2 (Continued)

Sr. No.	Parts of plant	Name of compound	CID number	Structural formula	Affinity (kJ/mole)
		Benzene, 2-methyl-1, 3, 5- trimethyl	11084258		−5.8
		Nonanedioic acid, dimethyl ester	15612		−6.1
		Tridecanoic acid, methyl ester	15608		−6.7
		n-Hexadecanoic acid	985		−7.7
		Octadecanoic acid, methyl Ester	8201		−8.1
		p-Hexyloxy nitro benzene	84912		−8.1
		-Sitosterol	457801		−8.1

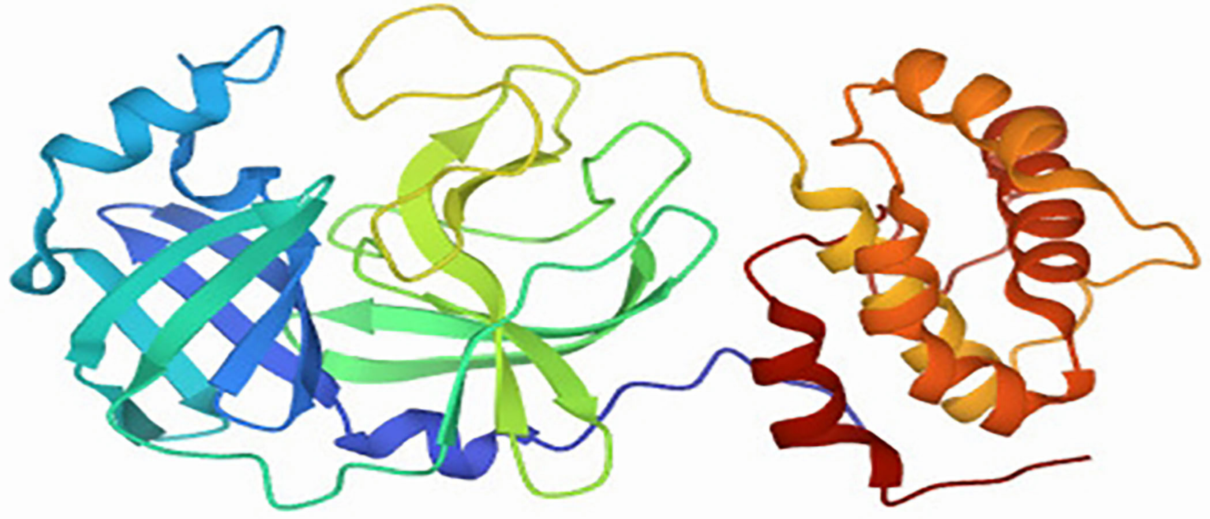


FIGURE 6
Three-dimensional structure of *6LU7* main protease (Mpro): [PDB accession ID: 002214U].

Arisaema genus (59). The Japanese encephalitis (JE) condition was caused by the Japanese encephalitis virus (JEV) and affects millions worldwide. The genus *Arisaema* was used to cure this deadly virus, by which nearly 70,000 people per year are affected (60). A methanolic extract of the roots of *A. jacquemontii* Blume was proven to have antiviral properties

TABLE 3 Summary of docking results of 22 compounds, functional residues involved in hydrophobic interactions, hydrogen bonds, and their binding affinities against *6LU7* target protein.

Sr. No	Parts of plant	Name of compound	Hydrophobic interactions	Total hydrogen bonds	Affinity kJ/ mole
1.	Leave	2-Flouoro-6-(trifluoromethyl)-acetophenone	Phe 219 (A) Trp 218 (A) Leu 271 (A) Gly 275 (A) Glu 270 (A) Asn 277 (A)	(1) Arg 279 O-NH2 = 2.89 Å	−7.4
2.	Pulp	Triallylmethylsilane		No results	
		Propane nitrile, 3- (methylthio)	Asn 151 (A) Phe 294 (A) Thr 292 (A) Gln 110 (A)	(2) Thr 111 N-N = 3.20 Å N-OG1 = 2.96 Å	−3.4
		Octane, 1-(propylthio)-	His 246 (A) Val 202 (A) Gln 110 (A) Pro 293 (A) Ile 249 (A) Phe 294 (A)	No	−5.1
3.	Seed	2, 5- dimethyl-3- isopropylpyrazine	Lys 5 (A) Phe 291 (A) Glu 288 (A) Arg 4 (A) Phe 3 (A) Leu 282 (A) Trp 207 (A)	No	−5.3
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	Gly 11(A) Lys 12 (A) Pro 9 (A) Ile 152 (A) Phe 8 (A) Arg 298 (A) Phe 294 (A)	No	−7.3
		Pentadecanoic acid, methyl ester	Gly 11 (A) Lys 12 (A) Pro 9 (A) Ile 152 (A) Phe 8 (A) Phe 294 (A)	No	−7.2
4.	Stem	Ortho-Methoxyacetophenone	Phe 294 (A) Thr 292 (A) Asp 295 (A) Gln 110 (A) Asn 151 (A)	(1) Thr111 O2-N = 3.23 Å	−5.8
		4'-Diethylaminoacetanilide	Phe 294 (A) Asn 151 (A) Gln 110 (A) Ile 106 (A) Val 104 (A)	(1) Thr111 O-N2 = 3.24 Å	−6.4

(Continued)

TABLE 3 (Continued)

Sr. No	Parts of plant	Name of compound	Hydrophobic interactions	Total hydrogen bonds	Affinity kJ/ mole
		Nonanoic acid, methyl ester	Phe 294 (A) Thr111 (A) Asn 151 (A) Ile 106 (A) Val 104 (A) Arg 105 (A) Asp 295 (A)	(1) Gln 110 NE2-O2 = 2.99 Å	−5.4
		2,5-Dimethylcyclohexanol	Leu 282 (A) Arg 4 (A) Glu 288 (A) Phe 291 (A) Trp 207 (A) Lys 5 (A)	(1) Phe 3 O–O = 2.98 Å	−5.4
5.	Rhizome	Beta, - methyl xyloside	Glu 270 (A) Trp 218 (A) Arg 279 (A) Leu 271 (A) Gly 275 (A)	(1) Phe 219 O5-O = 3.05 Å	−6.3
		Nonanoic acid	Glu 240 (A) His 246 (A) Pro 108 (A) Val 202 (A) Gln 110 (A) Phe 294 (A) Ile 249 (A)	No	−5.3
		3- decanol	Thr 292 (A) Gln 110 (A) Asn 151 (A) Phe 294 (A) Asp 153 (A)	(1) Thr 111 O–O = 2.85 Å	−5.8
		Phenol, 2, 5- bis (1,1-dimethylethyl)-	Gly 11(A) Lys 12 (A) Pro 9 (A) Ile 152 (A) Phe 8 (A) Arg 298 (A) Phe 294 (A)	No	−7.3
		Benzene, 2-methyl-1, 3, 5- trimethyl	Gln 110 (A) Phe 294 (A) Asn 151 (A) Ile 152 (A)	No	−5.8
		Nonanedioic acid, dimethyl ester	Leu 271 (A) Tyr 239 (A) Leu 286 (A) Leu 287 (A) Thr 199 (A) Asp 289 (A) Arg 131 (A)	No	−6.1

(Continued)

TABLE 3 (Continued)

Sr. No	Parts of plant	Name of compound	Hydrophobic interactions	Total hydrogen bonds	Affinity kJ/ mole
		Tridecanoic acid, methyl ester	Glu 240 (A) Gln 110 (A) Val 202 (A) Pro 293 (A) Phe 294 (A) Ile 249 (A)	(1) His 246 NE2-O2 = 3.05 Å	−6.7
		n-Hexadecanoic acid	Ile 152 (A) Pro 9 (A) Phe 294 (A) Phe 8 (A) Arg 298 (A)	(2) Gly 11 Lys12 N-O2 = 3.15 Å N-O2 = 3.18 Å	−7.7
		Octadecanoic acid, methyl ester	Glu 240 (A) His 246 (A) Pro 108 (A) Val 202 (A) Gln 110 (A) He 249 (A) Phe 294 (A) Pro 293 (A)	No	−8.1
		p-Hexyloxy nitro benzene	Gly 11 (A) Lys 12 (A) Pro 9 (A) He 152 (A) Phe 8 (A) Arg 298 (A) Phe 294 (A)	No	−8.1
		Gamma-Sitosterol	Gly 275 (A) Leu 287 (A) Tyr 239 (A) Thr 199 (A) Asp 289 (A) Glu 288 (A) Leu 286 (A) Lys 137 (A)	(2) Leu 271 Leu 272 O–O = 3.03Å O–O = 3.09 Å	−8.1

(17). The GC-MS results of *A. jacquemontii* Blume showed the presence of 22 compounds in it. All the compounds have a good binding affinity as shown after molecular docking. Only one compound trimethylsilane revealed no docking results because Si is a sensitive case in docking. While, according to Kant et al. (59), only three compounds were observed from *Arisaema* that are 2-hydroxydiplopterol, 30-nor-lanost-5-ene-3β-ol and 30-nor-lanost-5-ene-3-one and out of all these three compounds only one compound showed docking results with−3.83 binding affinity and no results were shown by rest of the two compounds. GC-MS analysis of methanolic extracts of Salacia oblonga roots revealed the presence of compounds such as n-hexadecanoic acid (11.94), 6-octadecanoic acid (2.24), hexadecanoic acid, 3-hydroxy methyl ester (1.84), n-methoxy-n-methylacetamide (17.38), phytol (0.58), 1, 2 benzene dicarboxylic acid (61). While GC-MS identified the same chemicals in *A. jacquemontii* Blume with variable binding affinity, indicating that they might be used to treat COVID-19.

The study could add to the expanding body of evidence that the Arisaema genus has antiviral properties, owing to phytoconstituents produced from these medicinal plants' ability to inhibit viral mediators such as HSV. Molecular docking is one of the most effective approaches for creating novel ligands for proteins with specified functions, and it is widely utilized in drug development. The antiviral activity of *Arisaema*, a plant from the Araceae family, was tested by docking phytochemical compounds with the *6LU7* protein to see if they might attach to the enzyme's active site and prevent it from working. Antiviral proteins were docked against these 22 compounds to see how much binding impact they had on finding a new drug or inhibitor to limit or decrease disease infection. Each molecule was docked using topoisomerase IV type B and spectroscopically identified using GC–MS. Swiss PDB Viewer identified the active site additives as Leu 209, Thr 207, Arg 176, Val 160, Leu 135, Ile 134, Asp 121, Pro 119, Met 118, Gly 117, Arg 116, Asp 113, Glu 90, Asp 89, Val 88, Ser 87, Asn 86, Asp 85, and Val 83 and used −8.5 kcal/mol energy (62–64). Similarly, 8.1 kJ/mol affinities were found in two *A. jacquemontii* Blume rhizome's compounds, namely, p-hexyloxy nitrobenzene and gamma-sitosterol, whereas the first compound had no hydrogen bonds and the second had two hydrogen bonds, O-O and O-O with bond distances of 3.03 A and 3.09 A, respectively (Table 3).

Docking analysis revealed that nelfinavir forms H-bonds with the *6LU7* amino acids Glu 166, Gln 189, and Gln 192 obtained from different medicinal plants in the tropical region (65). However, nonanoic acid, methyl ester, also forms a hydrogen bond with Gln 110, and this compound was obtained from the methanolic extract of the stem of *A. jacquemontii* Blume. The hydrophobic interactions obtained through ligand–protein interaction are Phe 294 (A), Thr 111 (A) Asn 151 (A), Ile 106 (A), Val 104 (A), Arg 105 (A), and Asp 295 (A). The amino acid residue interactions at the active site of COVID-19 Mpro are predicted to be mediated by hydroxy groups (-OH), ketone groups (=O), and ether groups (-O-) in luteolin and kaempferol compounds. The same bonding groups were observed in Mpro protein with different compounds of *A. jacquemontii* Blume such as 2,5-Dimethylcyclohexanol, beta-methyl xyloside, 3-decanol, and gamma-sitosterol with binding affinities −5.4, −6.3, −5.8, and −8.11, respectively (65).

COVID-19 has stressed our healthcare system and underlined the essential role of laboratory medicine and herbal medicine in tackling the spread of new transmissible agents. Networks of COVID-19 laboratories have been set up to support the specific needs of citizens and patients, and they will continue to be fundamental during the restarting of social and work activities (66, 67). Plants, therefore, play an important role in its cure because different herbal teas were considered effective in treating this deadly disease (68, 69). This study helped us to discover an inhibitor against protease so that it can be helpful in the cure of COVID-19. To our knowledge, there is currently no study on the COVID-19 infection in terms of *A. jaquemontii*, although scientists are working on it. After comprehensively reviewing the literature and implementing our own research, we observed that *A. jacquemontii* Blume has a high potential for combating COVID-19.

This study satisfies a number of the sustainable development goals (SDGs) by playing a key role in fulfilling these goals. It helped us to maintain life on land. Because this is a medicinal plant and is used to treat several diseases not only in humans but also in animals. After knowing the importance of this medically important species, our utmost desire should be to support the local community in its consumption and production so that it can be further utilized to treat many disorders. As its consumption and production were supported by the local community, it opened up a number of livelihoods for the poor, which will provide income resources for the community group.

Conclusion

A total of 22 compounds were extracted from *A. jaquemontii* from five different parts of the plant, including seed, stem, pulp, leaf, and rhizome. The antiviral activity of these 22 potential drugs was studied *via in silico* characterization. The *6LU7* Mpro protein has been identified as a key and effective target for inhibiting the new COVID-19. Of all these chemicals, two had the highest binding affinity of −8.1 kJ/mol. Based on these selected compounds' intermolecular interaction and binding energy, it has also been concluded that these chemical compounds could be a potential drug for SARS-CoV-2 infection. Because there is no experimental technique in *in silico* characterization using molecular docking, it explains the potential of isolated chemicals through different software. These isolated chemicals have such high binding energy values that they can be further processed for experimental purposes, which will be another study and more support for this effort. It could be an effective drug by blocking the replication in maturity of the virus in the host body.

Recommendations

These findings focus on bioactive phytochemicals synthetically modified to their structural motifs that could be potentially used against infectious diseases. This study's findings might be utilized to generate additional phytochemicals to be used against COVID-19 in the future. The docked compounds are recommended for *in vitro* and *in vivo* studies in the future to possibly overcome the infectious viral diseases of humans as well as animals.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, and writing—original draft preparation: SS, SK, GM, AA, IK, and ZA. Writing—review and editing: SS, SK, GM, AA, IK, ZA, HH, JP, JY, and AR. Visualization: SS, SK, GM, AA, IK, ZA, and AR. Supervision: SK, GM and AR. Project administration: SK, GM, HH, JP, JY, and AR. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.964741/full#supplementary-material

SUPPLEMENTARY TABLE 1
Three dimensional images showing protein - Ligand complex, bond present, and different hydrophobic interactions.

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The significant association between quick return and depressive symptoms and sleep disturbances in paid workers: A nationwide survey

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Objectives: Although many studies have examined the association between shift work and depression or insomnia, few studies have examined the relationship between quick return (QR) to work and depressive symptoms, regardless of shift work. Thus, in this study, we aimed to assess the association between depressive symptoms (DS)/sleep disturbances (SDs) and QR.

Methods: Data from the 6th Korean Working Conditions Survey (2020) were used for this study. Paid workers aged between 20 and 65 years were included. DS were defined using the World Health Organization Well-Being Index (WHO-5) with a cut-off 50, and SD was defined as the occurrence of the following symptoms several times per month: difficulty in falling asleep, waking up in the middle of the night, or feeling tired even after waking up. QR was defined as "at least one case where the working interval between leaving work and the next day's work was <11 h in the past month." Multivariable logistic regression was performed to estimate the adjusted odd ratios (aORs) and 95% confidence intervals (CIs). Mediation analysis was conducted to examine whether SD was a significant mediator in the association between QR and DS.

Results: Among the 27,554 participants, DS occurred in 8,277 patients, while SD occurred in 6,264 patients. The aORs (95% CIs) of DS and SD by QR were 2.01 (1.78–2.27) and 3.24 (2.87–3.66), respectively, after adjusting for age, gender, income, education, working hours, job status, working duration, region, shift work, and occupation. SD was a significant mediator in the association between QR and DS.

Conclusion: QR is significantly associated with DS or SD regardless of demographic factors and the working environment. The significant relationship between QR and DS may be mediated by SD.

KEYWORDS

quick return, depressive symptoms, sleep disturbances, paid workers, mediation

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Introduction

Depression, also known as major depressive disorder, is a widespread and notable medical ailment that can negatively affect one's feelings, thoughts, and behaviors. Depression symptoms include impaired attention, feelings of overwhelming guilt or low self-worth, despair about the future, thoughts of death or suicide, interrupted sleep, food- or weight-related changes, and feelings of extreme weariness or lack of energy (1).

As a debilitating disorder, depression can negatively affect people and their relationships with family and friends, work, sleeping and eating habits, and overall health (2). In 2019, depressive disorder prevalence worldwide was 5.02% for those aged 20 years or older and 4.75% for those aged between 20 and 54 years (3).

Before the coronavirus pandemic in South Korea (henceforth Korea), 19.8% of general health checkups in 2019 involved individuals with depressive symptoms (DS) (4), and the 2018 National Health and Nutrition Examination Survey showed that 21.0% of individuals were sufferers of DS (5). In Korea, Shin et al. (6) showed that only 4.17% of patients receive treatment for depression and that the estimated cost of such treatment was 3,461 USD per patient. Furthermore, depression is a contributing factor for both absenteeism and reduction in worker productivity (7, 8). Due to Coronavirus disease 2019 (COVID-19), the prevalence of depressive symptoms in Korea was 30.7% by strong association with changes in sleep pattern (9).

The prevalence of depressive symptoms or depression was considerably high in various occupational fields and nations. Approximately 20% of American social workers have been found to be on antidepressant medications, and 60% were found to have experienced depression in the past (10). Moreover, according to the multi-national meta-analysis including Asia countries reported that 28.8% of physicians were found to have suffered from depression (11). In addition, Western countries with similar role stress for nurses showed similar results (12, 13). The prevalence of depressive symptoms in Korean workers was also estimated by 39% based on the WHO-5 well-being index, with the highest prevalence of depressive symptoms in economic sector (14). Further, 21.0% of industrial workers were found to suffer from depression with similar estimated prevalence across the continents (22% for Asia, 18% for Europe, 20% for America)—a higher prevalence than that found among the general population (15). As such, many workers worldwide have complained about suffering from depression.

Contributing factors for depression among workers included sex, marital status, religious beliefs (16), disease (17, 18), diet, less leisure time (18, 19), shift work (20), long working hours, and lower salary (18). A study on depression among Korean workers found that depression was related to work stress (long working hours, making influence decisions, and work pressure) (21); furthermore, beyond work stress, job insecurity was also found to have an impact on depression (22).

Besides these factors, insomnia has been shown to be an important predictor of depression (23). Previous studies found that the improvement in insomnia is significantly associated with a decreased risk of depression (24, 25). Moreover, since quick return (QR) had significant association with higher risk of sleeplessness, sick leave, and injury in the previous studies (26–29), a study regarding the relationship or mediating effect between QR, insomnia, and depression might be needed. Furthermore, although many studies have examined the relationship between shift work disorder and depression, few studies have examined the relationship between DS and QR regardless of shift work.

Therefore, this study aims to investigate the relationship between DS/sleep disturbances (SDs) and QR independent of demographic and working conditions. Moreover, we aimed to establish the association between DS and QR while considering any indirect effects of SD.

Methods

Data sources and study population

This cross-sectional study utilized data from the Korean Working Conditions Survey [Sixth Korean Working Conditions Survey (KWCS), 2020], which has been conducted by the Occupational Safety and Health Research Institute since 2006. The study, which utilized questionnaires based on the European Working Conditions Survey, interviewed workers aged $\geq$ 15 years, who formed the study sample (30). The study participants were chosen randomly to represent the working population in Korea. The questionnaires included items regarding occupational classification, type of work, employment status, hazardous exposures, working profiles including working hours, QR, night shifts, and mental health effects of the participants.

Among 50,538 participants, 33,063 paid workers were initially enrolled in this study. Participants aged < 20 or > 65 years ($n = 3{,}556$) and those with missing values ($n = 1{,}953$) were excluded. The missing values included those related to working hours ($n = 200$), working years ($n = 269$), shift work ($n = 81$), QR ($n = 150$), SD ($n = 19$), DS ($n = 42$), income ($n = 1{,}177$), and education ($n = 15$).

This study was conducted according to the ethical requirements of the 1975 Declaration of Helsinki and was approved by the Institutional review board of Severance Hospital of the Yonsei University Health System (IRB No. 4-2021-1283). Informed consent was waived owing to the retrospective nature of this study.

Variables and outcomes

This study's primary outcomes involved DS. The depressive group was defined using the World Health Organization Well-Being Index (WHO-5), which is used worldwide for assessing subjective well-being (31) (cut-off score: < 50) (32). The QR group was defined as participants who responded "yes" to the questionnaire item, "Has there been at least one case in the past month where the interval between leaving work and the next day's work was <11 h?" The secondary outcome of this study involved SD. The questionnaires defined SD as the presence of any of the following symptoms at least several times per month: difficulty in falling asleep, waking up in the middle of the night, and feeling tired even after waking up.

Age and gender were used based on the information of conducted survey and age was utilized as a continuous variable. Education level was classified into three groups based on participants' responses: high school graduate or under, college graduate, and university graduate. Income level was classified into three groups according to income per month: < 2 million KRW, 2 to < 4 million KRW, and ≥ 4 million KRW. Working hours were estimated for each week and stratified into three groups: ≤ 40 h, 41–51 h, and ≥ 52 h. Working duration was stratified into five groups: none, < 5 years, 5 to < 10 years, 10 to < 20 years, and ≥ 20 years. Employment type was classified into three groups: full-time, part-time, and temporary workers. Occupational classification was stratified into three groups: white collar (managers, professionals and related workers, and clerks), blue collar (craft and related trades workers, equipment workers, machine operating and assembling workers, and elementary workers), and others (service workers, sales workers, and skilled agricultural, forestry, and fishery workers). Shift work was defined as "yes" or "no" based on the response of the related question "I do shift work." Central and rural regions were defined based on the residential area of the participants.

Statistical analyses

The baseline characteristics of the participants stratified according to QR were compared using chi-squared test and independent *t*-test for categorical and continuous variables, respectively. Multivariable logistic regression modeling was sequentially performed in order to estimate the adjusted odds ratios (ORs) and confidence intervals (CIs) of DS and SD based on QR. Model 1 was adjusted by age and sex. Model 2 was adjusted by age, sex, income, and education level. Finally, model 3 was adjusted by working hours, working duration, employment type, occupational classification, region, and shift work in addition to covariates used in model 2. Subgroup analysis stratified by gender was further conducted along with multivariable logistic regression models. Moreover, interaction analysis was implemented in order to examine the interactions between long working hours and QR. Furthermore, mediation analysis was conducted in order to examine whether SD were a significant mediator in associating QR with DS. Mediation analysis was performed according to the Baron and Kenny method, and the Sobel test was performed (33).

Regarding sensitivity analyses, propensity score matching (PSM) (ratio: 1:3) was performed using the nearest neighbor method (caliper width: 0.1); the matching variables were age, gender, income, education level, working hours, working duration, employment type, occupational classification, region, and shift work. The absolute mean standardized difference was calculated for each variable to evaluate the PSM balance. The baseline characteristics of the participants, which were stratified using QR in the matched cohort, were compared using a chi-squared test and an independent *t*-test. Furthermore, multivariable logistic regression modeling was conducted, and the different cut-off of WHO-5 index score (≤28) was applied for depression as a sensitivity analysis (34, 35). Adjusted ORs (95% CIs) of depression were estimated for workers with a working duration encompassing more than 1 year.

All statistical analyses were two-sided, and $p < 0.05$ was considered significant. This study used R software version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) to perform all statistical analyses.

Results

Baseline characteristics of the participants

Among the 27,554 participants, the mean (standard deviation) age was 43.7 years (11.7), and 77.3% of the participants were men. Depression occurred in 8,277 participants (30.0%) [7,731 [29.3%] in the non-QR group and 546 [45.2%] in the QR group]. The baseline characteristics of the participants were stratified using QR, as summarized in Table 1. The QR group had a significantly higher prevalence among workers who had the following characteristics: young age, male, full-time, long working hours, long working duration, SD, high education level, high income level, central region, shift work, and depression (compared to the non-QR group [all $p < 0.05$]). Moreover, regarding the prevalence of DS and SD stratified by sex, women group has a significantly higher prevalence of DS and SD compared to men group [1983 [31.8%] vs. 6294 [29.5%] for DS, 1519 [24.3%] vs. 4745 [22.3%] for SD, respectively, all $p < 0.001$].

Relationships among QR, DS, and SD

The association of QR with DS and SD was significant in the univariable logistic regression with crude ORs (95% CIs) of 1.98 (1.77–2.23) and 3.53 (3.15–3.97), respectively. After

TABLE 1 Baseline characteristics of the participants (*n* = 27,554) in 6th KWCS (2020) stratified by quick return.

Variable	**No quick return (*n* = 26,345)**	**Quick return (*n* = 1,209)**	***p*-value**
Age			0.01
Mean (SD)	43.68 (11.70)	42.87 (10.66)	
Gender			<0.001
Women	6,039 (22.9%)	203 (16.8%)	
Men	20,306 (77.1%)	1,006 (83.2%)	
Job status			<0.001
Full-time	21,589 (81.9%)	1,075 (88.9%)	
Part-time	3,411 (13.0%)	100 (8.3%)	
Temporary	1,345 (5.1%)	34 (2.8%)	
Working hours			<0.001
≤40	18,887 (71.7%)	536 (44.3%)	
41–51	5,260 (20.0%)	370 (30.6%)	
≥52	2,198 (8.3%)	303 (25.1%)	
Working duration			<0.001
<1	4,274 (16.2%)	137 (11.3%)	
1 to <5	9,995 (37.9%)	393 (32.5%)	
5 to <10	5,629 (21.4%)	309 (25.6%)	
10 to <15	4,449 (16.9%)	262 (21.7%)	
≥15	1,998 (7.6%)	108 (8.9%)	
Sleep disturbance			<0.001
No	20,676 (78.5%)	614 (50.8%)	
Yes	5,669 (21.5%)	595 (49.2%)	
Education			<0.001
High school graduate or under	10,889 (41.3%)	431 (35.6%)	
2-year college	5,254 (20.0%)	199 (16.5%)	
Over university	10,202 (38.7%)	579 (47.9%)	
Income (10,000 KRW)			<0.001
<200	7,320 (27.8%)	158 (13.1%)	
200 to <400	15,110 (57.4%)	751 (62.1%)	
≥400	3,915 (14.8%)	300 (24.8%)	
Region			<0.001
Rural	12,994 (49.3%)	554 (45.8%)	
Central	13,351 (50.7%)	655 (54.2%)	
Occupation			<0.001
White collar	12,419 (47.2%)	595 (49.2%)	
Blue collar	7,331 (27.8%)	411 (34.0%)	
Others	6,595 (25.0%)	203 (16.8%)	
Shift work			<0.001
No	23,994 (91.1%)	847 (70.1%)	
Yes	2,351 (8.9%)	362 (29.9%)	
Depressive symptoms			<0.001
No	18,614 (70.7%)	663 (54.8%)	
Yes	7,731 (29.3%)	546 (45.2%)	

KRW, Korean won; KWCS, Korean Working Conditions Survey; SD, standard deviation.

adjusting the covariates in the multivariable logistic regression models, the adjusted ORs (95% CI) of DS and SD by QR were 2.01 (1.78–2.27) and 3.24 (2.87–3.66), respectively, in the final model (Table 2). In the stratification analysis, the adjusted ORs (95% CIs) of DS and SD by QR in the final model were 2.12 (1.86–2.43) and 3.39 (2.96–3.87), respectively in men and 1.58 (1.18–2.12) and 2.62 (1.96–3.51), respectively in women; all of these were significant (Table 2). The interaction analysis results for QR, working hours, and depression are shown in Figure 1. Adjusted ORs (95% CIs) of DS and SD based on "QR & working hours ≥40 group," "QR & working hours <40 group," and "non-QR & working hours ≥40 group" were 2.55 (2.18–2.99), 1.93 (1.61–2.30), and 1.17 (1.10–1.25), respectively for DS (Figure 1A) and 4.43 (3.78–5.20), 3.29 (2.75–3.93), and 1.35 (1.26–1.44), respectively for SD (Figure 1B), compared to the "non-QR & working hours <40 group." When mediation analysis was performed with SD as a mediator between QR and DS, because the direct effect (1.183) was less than the total effect (1.902), SD was found to be a partial mediation with partial interaction effects (z value of Sobel test: 19.3202) (Figure 2).

Sensitivity analyses

After propensity matching (ratio: 1:3), 4,790 patients remained in the analysis. The baseline characteristics between the QR and non-QR group were well-balanced according to the absolute standardized mean difference below 0.1 (Supplementary Table S1). Multivariable logistic regression model analysis showed that the association of DS with QR was statistically significant [final model; adjusted OR [95% CI] 1.97 [1.72–2.25]]. Moreover, after using different cut-off scores ≤ 28 in the WHO-5 index, the result found a significant association with QR and DS [adjusted OR [95% CI] 2.25 [1.94–2.62] in the final model]. Additionally, analysis involving participants who worked for more than 1 year showed significant results [adjusted OR [95% CI] 1.99 [1.75–2.27] in the final model]. All the results related to sensitivity analyses are summarized in Supplementary Table S2.

Discussion

This study used nationwide survey data to examine the significant association between QR and DS and SD. A multivariable logistic regression model analysis with adjustments of age, gender, income, education, working hours, SD, job status, working duration, region, shift work, and occupation confirmed the significant association of DS and SD with QR. Stratification analysis showed more predominant associations between depression and QR in men.

TABLE 2 Adjusted ORs (95% CIs) of depressive symptoms and sleep disturbance by quick return with stratification of gender in a logistic regression model.

	Gender	Quick return	Crude model	Model 1	Model 2	Final model
Depressive symptoms	Total	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	1.98 (1.77–2.23)	2.04 (1.81–2.29)	2.17 (1.93–2.44)	2.01 (1.78–2.27)
	Male	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	2.11 (1.86–2.39)	2.15 (1.89–2.44)	2.27 (2.00–2.59)	2.12 (1.86–2.43)
	Female	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	1.54 (1.16–2.05)	1.57 (1.18–2.09)	1.72 (1.29–2.30)	1.58 (1.18–2.12)
Sleep disturbances	Total	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	3.53 (3.15–3.97)	3.67 (3.26–4.13)	3.70 (3.28–4.16)	3.24 (2.87–3.66)
	Male	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	3.71 (3.26–4.21)	3.80 (3.34–4.32)	3.86 (3.39–4.39)	3.39 (2.96–3.87)
	Female	No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
		Yes	2.97 (2.24–3.94)	3.09 (2.33–4.10)	3.00 (2.25–3.98)	2.62 (1.96–3.51)

Model 1: adjusted by age.
Model 2: adjusted by age, income, and education.
Final model: adjusted by age, gender, income, education, working hours, sleep disturbance, job status, working duration, region, shift work, and occupation, except for each stratification variable.
OR, odds ratio; CI, confidence interval.

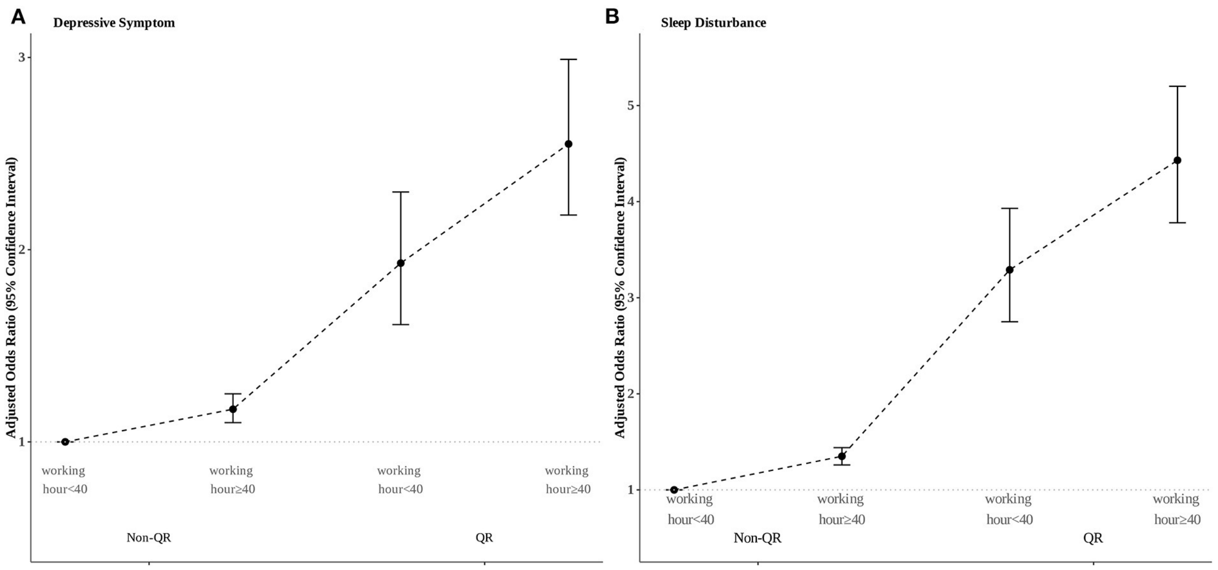

FIGURE 1
Interaction analysis of long working hours and quick return on **(A)** depressive symptoms and **(B)** sleep disturbance.

Moreover, interaction analysis involving working hours showed a synergetic interaction between long working hours and QR regarding DS and SD. The mediation analysis demonstrated that SD was a mediator in the association between QR and DS. Finally, sensitivity analyses with PSM, using different cut-offs for the WHO-5 index score, and analysis of workers with working duration ≥ 1 year presented similar significant results.

Previous studies on QR and mental disorders (including insomnia, anxiety, and depression) mainly focused on individuals who performed shift work, especially health care workers such as nurses (27, 36, 37). These past studies presented insignificant associations between depression and QR and significant associations between sleep problems and QR. Unlike these past studies, our study found that QR was significantly related to both DS and SD. This result can be attributed to several reasons. First, our study included various occupations (not only health care workers) and identified a significant relationship between QR and DS/SD regardless of shift work and other working profiles. The effect of shift work may be underestimated due to the healthy shift worker

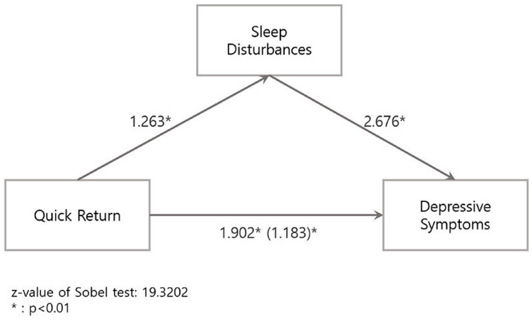

FIGURE 2
Mediation analysis of sleep disturbances on the association between quick return and depressive symptoms.

effect, which involves a selection of shift work based on pre-employment screening and other health problems (38); it implies the underestimation of the effect of QR on the outcomes in the previous studies. Indeed, our study results showed that shift work was not significantly associated with DS (Supplementary Table S3) but significantly associated with SD. Further studies should consider the healthy worker effect, which this study could not clarify due to its limited retrospective cross-sectional design. Second, unlike previous studies, our study recruited a large number of participants. Because of the sufficient study sample size, our results may have sufficient statistical power.

In the final multivariable logistic regression model, the magnitude of the association between QR and DS/SD was found to be prominent, compared to other covariates (Supplementary Tables S3, S4). QR may be an important factor for depression or insomnia regardless of demographic characteristics and working conditions. Previous study conducted by Kim et al. reported high prevalence of depressive symptoms in Korea (30.7%) and the strongest factor for depression was changes in sleep pattern (9). Therefore, workers with QR, which is highly associated with sleep disturbance, were more likely to be vulnerable to the changes in sleep pattern from the COVID-19 situation. Additionally, interaction analysis found that long working hours and QR had a synergistic interaction with regard to DS and SD based on the synergy index suggested by Rothman et al. (39). This could be one possible explanation for the pronounced association of QR with DS and SD in men (compared to women), since male workers presented a significantly higher prevalence of long working hours and QR (Figure S1). Our study results thus suggest that male workers with long working hours and QR may be considered high-risk of DS and SD. Furthermore, given significantly higher prevalence of SD and DS in women group according to the result of our study as well as previous studies (40, 41), female workers should also be cautiously screened regarding mental health.

Sleep deprivation may be one of the most crucial factors for explaining the association of QR with SD and DS. Several studies have found that QR can shorten sleep duration and cause disrupted and restless sleep (most notably in the shift work system) (42–45). QR may also lead to circadian rhythm disturbances (just like in shift work). SD originating because of QR may form a mediator for the association between QR and depression, as suggested by the mediation analysis results in this study. Considering the plausible mechanism, which is independent of shift work, it may be necessary to conduct further in-depth study on the mechanisms of the QR group among regular workers regarding mental health.

Our study has several strengths. First, the sample utilized in this study was large and encompassed nationwide representative data, which have been frequently validated by other studies. Second, to our knowledge, this study is the first to elucidate the association between QR and depression in workers with various occupation and shift works, while most past studies have emphasized the association of QR with depression in shift workers or health care workers. Third, we employed several statistical methods to examine the association between QR and depression. Moreover, we utilized stratification and interaction analysis to examine vulnerable populations related to QR. However, this study also had several limitations. First, being a cross-sectional study, it could not examine the causal relationship between QR and depression. Further studies with well-designed cohorts should be implemented to clarify the association between QR and risks of developing depression. Second, since this study employed retrospectively collected data, there was a lack of information regarding medical records related to depression and exact frequency of QR occurrence, which could function as unmeasured confounders. Finally, there is a lack of study on the validation of the WHO-5 index among Korean workers, despite its frequent utilization in previous studies (14, 46, 47). Further studies should be conducted to elucidate the validity of WHO-5 index among Korean workers.

In conclusion, our study showed that QR is significantly associated with DS regardless of demographic factors and the working environment. The significant relationship between QR and DS may be mediated by SD. Given the high magnitude of this association presented with high adjusted ORs, workers with QR, in general, should be carefully managed and screened with regards to the risk of developing depression and insomnia. Furthermore, employers and employees both should attempt to keep 11-h uninterrupted recess between work for maintain proper mental health status, accompanied by more detailed policy establishment.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number (s) can be found below: https://oshri.kosha.or.kr/oshri/researchField/workingEnvironmentSurvey.do.

Ethics statement

The studies involving human participants were reviewed and approved by Severance Hospital's Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JS and BY wrote the manuscript and were responsible for the conception of the study and data analyses. JO and YK collected data and had processed data. J-HY and BY conceived the ideas. BY, JS, and J-HY contributed with insight, scientific discussion, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.990276/full#supplementary-material

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Use of *Parthenium hysterophorus* with synthetic chelator for enhanced uptake of cadmium and lead from contaminated soils—a step toward better public health

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Parthenium hysterophorus L. is a vigorous plant species with cosmopolitan distribution. It can uptake considerable quantities of heavy metals from the soil and accrue these metals in its different tissue. The use of chelating agent i.e., Ethylenediaminetetraacetic acid (EDTA) can boost up metal uptake capacity. Pot experiment was performed to evaluate phytoextraction potential of *P. hysterophorus* for lead (Pb) and cadmium (Cd) with and without the aid of EDTA chelator. Shoot length, weight of root and shoot (both fresh and dry), leaves number, and chlorophyll contents of *P. hysterophorus* got reduced with an increase in metal uptake. The results revealed the highest concentration of Cd in shoot without and with EDTA was 283.6 and 300.1 mg kg^{-1}, correspondingly. Increase in Pb concentration was also boosted up by the EDTA from its maximum concentration in shoot 4.30–9.56 mg kg^{-1}. Generally, Pb and Cd concentrations were greater in shoots of *P. hysterophorus* than the roots regardless of EDTA in the treatments. EDTA also impacted positively the accumulation of essential ions K^+, Na^+, and Ca^{+2} in *P. hysterophorus*. The capacity of *P. hysterophorus* to accumulate Pb and Cd found to be increased with EDTA in the soil. Bringing metals level in the soil in accordance to the WHO standards can improve the ecosystem as well as public health.

KEYWORDS

phytoextraction, *Parthenium hysterophorous*, cadmium, lead, EDTA, soil remediation, weeds, soil pollution

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Introduction

Heavy metals or potentially toxic elements have become a great risk to environmental safety due to continuous agricultural practices, industrial expansion and a rise in the population. Heavy metals do not decompose and as a result, they continue to build up in the environment (1). Application of fertilizers, sewage sludge, urban traffic, chemical emissions from industries and mining processes are the examples of anthropogenic sources (2). These appliances are responsible for increasing heavy metal concentrations in soil (3, 4). Heavy metals in agricultural lands and natural resources may pose a threat to public health due to their potential access to food chains (5–8). Constant application of pesticides and chemical fertilizers may build up the number of heavy metals in the soil (9, 10). Dry and wet waste residues from various point sources such as the steel industry, metal refineries, metal smelters, cement industries, and foundries also increase heavy metals to the soils. But mainly the combustion from engines using lead (Pb) improved petrol is liable for major production of Pb in soils adjacent to roads. According to some recent studies, vehicle exhausts are the biggest sources of Pb and Cd in the environment (11, 12).

Pb is a potentially toxic heavy metal above the permissible limits. Apart from public sewage sludge and leaded gasoline, it also results from mining, oils, paints, and unstable materials. Pb is widely used in many industrial applications as a tetraethyl Pb [$(CH_3CH_2)_4$ Pb], a longtime motor gasoline ingredient and a current stabilizer to certain petroleum, producing the most common heavy metal contaminants in the soil (13, 14). Increase in lead pollution contributes to soil toxicity and also disturb microbial diversity (15–17). According to the U.S. Environmental Protection Agency, 207,000 Pb-contaminated regions in the U.S. including millions of farms need to be cleaned (18). This dangerous pollutant could be taken up *via* plants and reaches the body by consumption of contaminated plant products and gathered in different organs. In severe cases, it may cause human mortality (19). Cd is an extremely cancer causing material that can trigger dangerous responses even in minute quantities (20). Cd can be taken up by plants and ultimately it gets transferred into the food chain (5). Like Pb, Cd also comes from both manmade and natural resources and has a major impact on the disruption of environmental quality. Road traffic could be a big source of Cd in adjacent areas as Cd on large scale is used in lead-acid batteries (12).

Many remediation techniques have been devised so far to handle the contaminated soils (21–23). In addition to traditional techniques of soil purification; phytoextraction is suitable for severely contaminated sites, while phytostabilization is widely used to remediate slight heavy metal contaminated soils (3, 24, 25). Phytoextraction is one of the phytoremediation type, in which the absorption and collecting of metals occur into plant aerial parts from polluted soil. Using plants that could bear a high level of heavy metals is crucial. Chelating substances have been used in metal contaminated soils as decontaminants to boost up phytoextraction lately (26). The cost of phytoextraction could be more than 10 times lower per hectare than standard soil remediation methods such as thermal soil remediation, air sparging and encapsulation. Phytoextraction can be implemented in all locations where soil and weather are appropriate for plant development (27). However, the capability of a plant to accumulate metals from the soil depends upon plant species and their growth habits. The plants selected for phytoextraction should have a rapid growth rate, more production of bio-mass, hyperaccumulater of heavy metal, broadly spread, stem to shoot translocation, toleration of toxic heavy metal impacts, pathogen and pest resistance, welladapted to environmental circumstances, simple to grow and harvest, and do not attract herbivores to prevent entering the food chain (28, 29).

One such plant is *Parthenium hyterophorus* L. This plant is preferred because of its rapid expansion and inedibility to herbivores. *P. hysterophorus* belongs to family Asteraceae, also recognized as congress grass, is an annual herb, invasive weed found across Pakistan and the world. It has been established to perform a fundamental function in the accretion of toxic metals particularly in contaminated sites (30). *P. hysterophorus* has a very high potential for remediating soils polluted with Cd and Pb (11). Phytoextraction employing *P. hysterophorus* is a cost-effective and possible remedy for the cleanup of Cd and Pb polluted soils. In Pakistan, this weed is scattering in harsh environments, despoiled areas, rocky crevices, across waterways, roads, and railway lines. It has also been recently identified in cultivated land.

While chelating substances are used as decontaminants in polluted soils to smooth the process of phytoextraction. Previous knowledge confirms the use of artificial metal chelates like EDTA in soils improved Pb uptake with the help of plants (31). EDTA produces soluble metal EDTA complexes, due to its strong affinity toward heavy metals, assisting in the solubility of soil metals and therefore improving metal accessibility to plants (32). However, EDTA is generally known for its excellent property of metal absorption in soil, but it is also toxic to some species and inhibits their growth. Additionally, EDTA has a weak biodegradation rate and stays for long time in environment (33). EDTA is so far the most proven and successful chelator for removing hazardous metals from soil (34). Some studies previously used phytoremediation for some other metals with or without using EDTA as a phytochelator, or they used some other plants/microbes as phytoremediators (35–38). It was hypothesized that the EDTA is the most potent chelator in lowering Pb and Cd bioaccessibility in soil (34). Both of these metals as well as *P. hysterophorus* are commonly found in the soils along roads. Keeping this information in mind, we aimed to study the metal uptake capability of this fast-growing weed (*P. hysterophorus*) with the help of EDTA

TABLE 1 Treatments used in set-1 and set-2.

Sets	Treatments	Pb ($mg\ kg^{-1}$)	Cd ($mg\ kg^{-1}$)	EDTA ($mmol\ kg^{-1}$)
Set-1	T0	—	—	—
	T1	1,000	—	—
	T2	2,000	—	—
	T3	2,000	—	5
	T4	1,000	200	5
Set-2	T0	—	—	—
	T1	—	200	—
	T2	—	400	—
	T3	—	400	5
	T4	1,000	250	5

chelator. This study used *P. hysterophorus* for the first time for its phytoremediation capabilities in combination with chelator (EDTA). Our objectives are (1) to use Pb and Cd simultaneously with EDTA to evaluate the phytoextraction potential of *P. hysterophorus*, whether the use of EDTA has any impact in decontaminating these metals by *P. hysterophorus* and (2) assess the effect of these combinations on growth and functioning of the plant.

Materials and methods

Experimental design

Clay pots of 30 cm in diameter were used in this Completely Randomized Designed (CRD) experiment. A total of 30 pots, filled with soil from Botanical Garden of GCWUS, were used in two sets of treatments assigning 15 pots for each treatment set. Ten kilograms of pure and dried sandy loam soil was filled up in each experimental pot. Seeds of *P. hysterophorous* were gathered from plants growing in non-contaminated areas and each container had ten seeds. *P. hysterophorous* doesn't require a lot of water to flourish due to its wild nature thus, tap water is provided twice a week in accordance with the plant's requirements. The temperature at the time of seed sowing was 36/20°C (day/night) and 24/12°C at the harvesting time period. After plant germination 2 sets of treatments each with 3 replicates were applied to the soils in the pots in 2 weeks (Table 1).

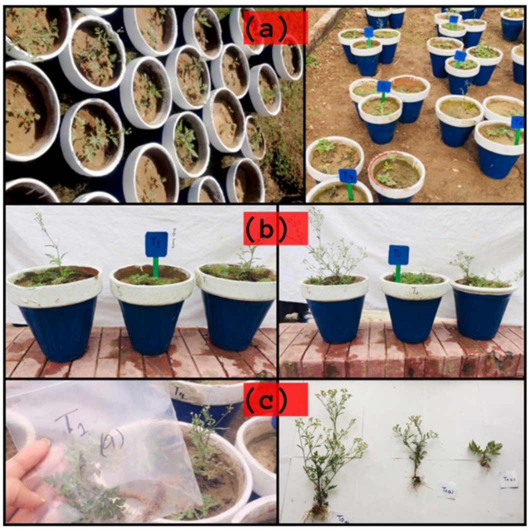

FIGURE 1
(a) Plants at seedling stage, **(b)** post treatment growth of seedlings, and **(c)** collection of plants for further processing.

Morphological attributes

Morphological parameters were studied in the laboratory after the collection of plant samples three months after the application of treatments. Their visuals attributes are given in Figure 1. Each plant sample was measured in terms of its height (cm), shoot length (cm), and root length (cm) with the help of a meter rod. Using digital balance, root and shoot fresh weights were measured in grams and the data were recorded. For dry weights, firstly, samples of shoots and roots have been dried for three days at 72°C in an oven. The dry weights of these samples were measured. Similarly, the total number of leaves in each pot was measured and the leaf area in each pot was measured following below formula:

$$\text{Leaf area} = (\text{Length} \times \text{Width}/\text{Total no. of leaves}).$$

Determination of photosynthetic pigments

The method of Arnon (39) was followed for the determination of photosynthetic pigments. Leaf samples were collected separately from each pot in triplicates. In total 0.2 g of fresh leaves of each sample were taken and ground well separately. Then the grounded samples were mixed up with 80% of acetone. Ten milliliter of each plant triplicates were made by mixing 80% of acetone and were placed in a dark place in the laboratory for 48 hours. Then the samples were run on a centrifuge machine to collect the supernatant which was then analyzed in a spectrophotometer (Hitachi, Model U2001, Tokyo, Japan). The absorbance of solution was measured at 480, 645, and 663 nm for carotenoids, chlorophyll a, and chlorophyll b, respectively. The following formulae were used:

$$\text{Carotenoids}\ (\text{mg/g f. wt.}) = [\text{Acar / EM}] \times 1000$$

$$\begin{aligned}
\text{Total Chl.(mg/gf.wt.)} &= [20.2(\text{O.D. }645) - 8.02(\text{O.D. }663)] \\
&\times V/1000 \times W \\
\text{Chl.a(mg/gf.wt.)} &= [12.7(\text{O.D. }663) - 2.69(\text{O.D. }645)] \\
&\times V/1000 \times W \\
\text{Chl.b(mg/gf.wt.)} &= [22.9(\text{O.D. }645) - 4.68(\text{O.D. }663)] \\
&\times V/1000 \times W \\
\text{Acar} &= \text{O.D. }480 + 0.114(\text{O.D. }663) \\
&- 0.638(O.D.\ 645) \\
\text{V} &= \text{Volume of the sample} \\
\text{EM} &= 2500 \\
\text{V} &= \text{Volume of the sample.}
\end{aligned}$$

Determination of heavy metals and ions

Heavy metals (Cd and Pb) and ions such as Na, K, and Ca concentrations in root and shoot samples were determined using the following procedure. Plant samples were collected and dried in an oven for three days at 72 °C, and then the dried-out material was ground into a powder with a pestle and mortar. For acid digestion, 0.2 g of dried material was taken in 100 ml sized beakers, and 20 ml of pure nitric acid was added and heated to 250°C on a hot plate. These beakers were removed from the hot plate and cooled down, when 10 ml of the solution remained then 10 ml of perchloric acid was added to these beakers and again heated on the hot plate until the contents became clear and fumes stopped evolving completely. Beakers were cooled down and filtered using filter papers (Whatman No. 2) and poured the solutions into cleaned sample bottles separately. Distilled water was added to each sample to make up a 100 ml solution. This extract was used for the determination of heavy metals and ions (40). Following acid digestion of the extracted samples, Atomic Absorption Spectrophotometer (Model: Perkin Elmer Analyst 100) was used to measure Na, Cd, and Pb concentrations, whereas flame photometer was used to measure Na and K concentrations (Model: Flame photometer 410, CORNING).

Translocation factor

A plant's ability to translocate heavy metals from roots to shoots and leaves was measured by its translocation factor (TF). Meanwhile, the shoot/root bioconcentration factors, translocation efficiency, and removal efficiency were calculated using these formulas (41, 42).

TF = the ratio of metal concentration in the shoot/metal concentration in the root.

Statistical analysis

The results were statistically examined using SPSS computer software's one-way analysis of variance (V 23). Non-significant was defined as a *P*-value greater than 0.05. The connection between Pb and Cd Translocation Factors in two sets of treatments was determined using a linear regression test.

Results and discussion

Determination of metals

P. hysterophorus accumulated higher concentration of Pb and Cd ($p < 0.001$) in roots and shoots (Tables 2, 3). The pattern of Pb and Cd accumulation was greater in the shoots than in the roots in all treatments (Figure 2). The maximum concentration of Pb was recorded in T4 in shoots as well as in roots (Figures 2A,B). In the case of Cd in set-2, maximum concentration (854.0 ± 25.2) was recorded in shoots in T3 (Figures 2C,D). However, T2 and T3 of set-2 show slight difference to each other, they differed highly significantly from the T0 though. In treatments T3 and T4, the improved accumulation of Pb and Cd in set-1 and set-2 respectively could be attributed to the addition of EDTA in these metal treatments. Our findings are consistent with prior studies, which indicated that EDTA had a considerable influence on the accumulation of Cd and Pb in plants (43, 44). A past study according to our findings reported that when the concentration of 0.25 mM EDTA was increased, fast absorption of Pb occurred in the shoot (45). One dosage of 10 mmol EDTA kg^{-1} enhanced. Ni, Cd, and Pb uptake in *Brassica rapa* and also increased their TF as there was a significantly larger concentration of these metals in upper plant parts as compared to the non-treated ones (46). The chelate-assisted phytoextraction technique appears to be more effective than a strategy for cleaning up Pb-contaminated soils that relies on the natural potential of some wild plant species (47, 48), these results have shown positive correlation with our study. The metal absorption with EDTA was also consistent with the findings of Madrid et al. (49), who found that EDTA was particularly effective at mobilizing metals from soil to the plant and can promote root-to-shoot translocation. Similarly, Turgot discovered in an investigation that 0.1 g/kg EDTA boosted total shoot: root translocation (50). According to (51), the addition of EDTA at rates of 2.5 or 5.0 mmol kg^{-1} considerably raised metal concentrations in plant shoots. High biomass plants may be useful for phytoextraction of heavy metals when exposed to large concentrations of chelate-solubilized

TABLE 2 ANOVA of various attributes of *P. hysterophorus* in response to Set-1 of treatments.

Source of variance	df	Root length	Shoot length	Root FW	Shoot FW	Root DW	Shoot DW	No. of leaves	Leaf area	Chlorophyll a	Chlorophyll b	Total chlorophylls	Carotenoids	Calcium (roots)	Calcium (shoots)	Potassium (roots)	Potassium (shoots)	Sodium (roots)	Sodium (shoots)	Pb (roots)	Pb (shoots)
Treatments	4	20.88**	234.4***	0.684**	37.97***	0.076***	0.602***	143.4***	0.033ns	0.184ns	0.043*	0.404ns	0.479***	12.15 ***	20.88***	5631***	3.721***	1256***	1720***	18.59***	39.23***
Error	10	3.030	11.82	0.110	1.425	3.968	0.001	9.466	0.010	0.081	0.011	0.149	0.034	0.064	0.034	4940	3546	1020	4666	0.083	0.183

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; ns, $P>0.05$.

TABLE 3 ANOVA of various attributes of *P. hysterophorus* in response to Set-2 of treatments.

Source of variance	df	Root length	Shoot length	Root FW	Shoot FW	Root DW	Shoot DW	No. of leaves	Leaf area	Chlorophyll a	Chlorophyll b	Total chlorophylls	Carotenoids	Calcium (roots)	Calcium (shoots)	Potassium (roots)	Potassium (shoots)	Sodium (roots)	Sodium (shoots)	Cd (roots)	Cd in (shoots)
Treatments	4	19.49**	237.6***	0.851**	45.32***	0.082***	0.607***	148.5***	0.048**	0.369***	0.078**	0.749***	0.624***	27.59***	111.3***	2.676***	8208***	1256***	14913***	2190.0***	3276***
Error	10	2.250	1.866	0.077	1.047	2.163	0.005	9.533	0.004	0.028	0.011	0.058	0.029	1.305	0.0513	3686	9133	1020	42000	14.63	432.9

***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; ns, $P>0.05$.

materials (51). In calcareous soils, EDTA gradually increases the mobility of Cd and Pb (52). EDTA-enhanced metal absorption by plant roots has already been documented in several prior studies (53–55).

Determination of ions

The ionic concentrations of K, Na, and Ca increased in roots and shoots with EDTA as compared to without EDTA treatments other than the control in both sets. In comparison to the control, T1 had the greatest fall in K level in both roots and shoots (Figures 3A,B). An increase in K in T3 and T4 compared to T1 might be related to EDTA in these treatments. In the case of Ca, T3 and T4 (with EDTA) showed maximum concentration whereas T1 and T2 (without EDTA) showed less concentration of Ca in the roots and shoots of both sets (Figures 3C,D). Nutrient ions take a vital role in cell turgor, plant development, and metabolism. Generally, lower growth rates in plants are caused by the deficiency of K inside the cells. The cytosolic roles played by the K cannot be fulfilled by other cations; hence a certain portion of plant biomass contains K (56). Ca ions are taken in by plants *via* non-selective channels in the cell membranes of their root systems. These non-specific channels also permit other divalent and some monovalent ions to pass through them (57). Ca is an important cell signaling component and helps the plants to get over various stresses such as temperature shock, changes in nutrient status, mechanical stimuli, pathogen attack, and drought (58). A lot of studies have reported the reduction in the concentration of K and Ca ions with increased concentration of Pb and Cd in the environment (59, 60). However, some studies, in accordance with our results, have reported an increase in plant K and Ca ions in the application of EDTA as compared to treatments where EDTA was not given (61).

The concentration of Na increased in both shoots and roots after applying treatments (Figures 3E,F). Na concentration in roots and shoots was found to be maximum in T4 when

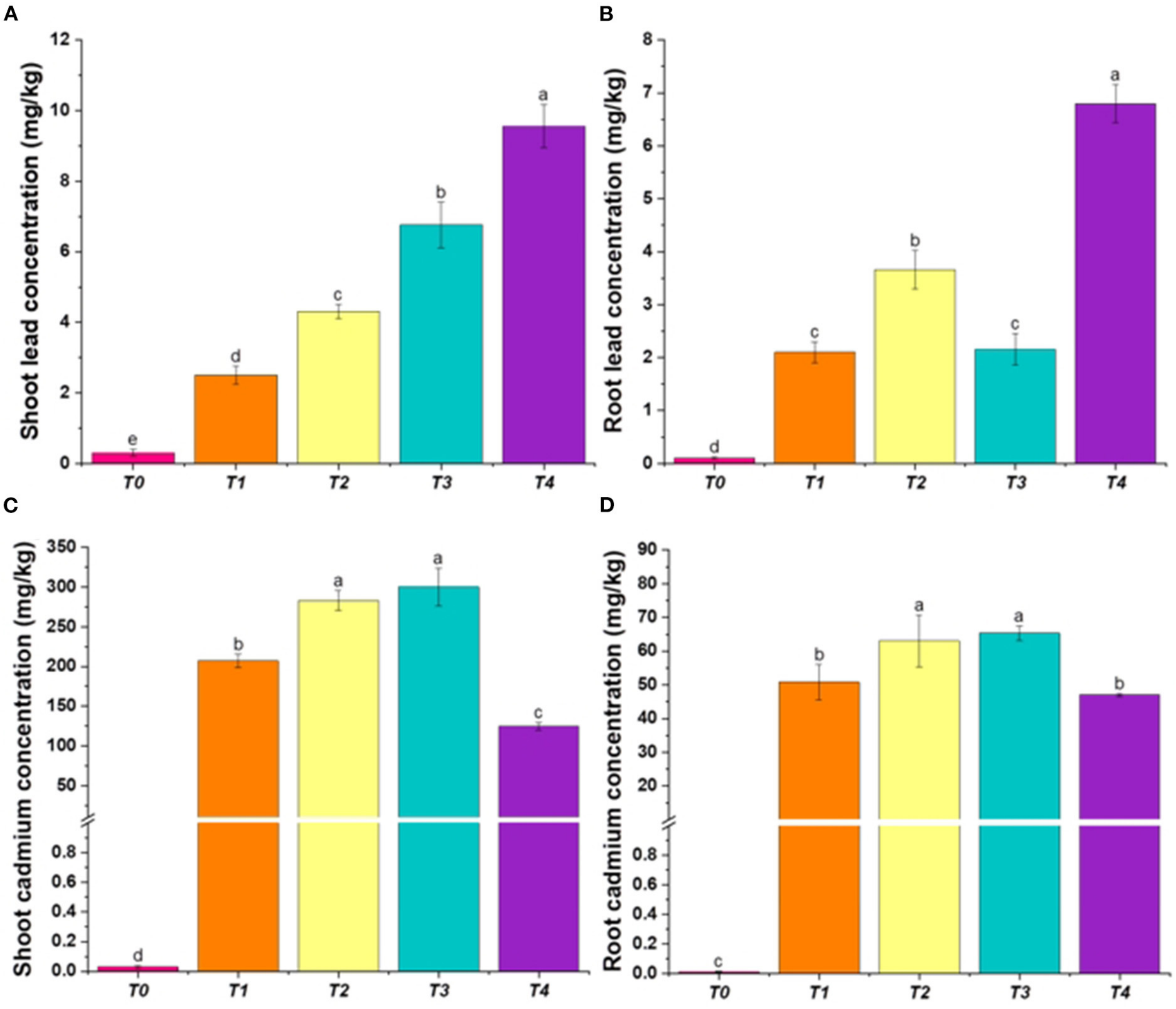

FIGURE 2
Heavy metals in *P. hysterophorus* under the effect of various treatments; **(A)** Pb concentration in *P. hysterophorus* shoot, **(B)** Pb concentration in *P. hysterophorus* root, **(C)** Cd concentration in *P. hysterophorus* shoot, and **(D)** Cd concentration in *P. hysterophorus* root.

compared to the T0. Thus, the results showed that in the presence of the EDTA plant ionic concentration increases as compared to the treatments where EDTA was not applied. Pb and Cd concentrations have reportedly been linked to an increase in Na concentration in *P. hysterophorus*.

We determined considerably high values of TF for both metals in both sets of treatments (Figure 4) which shows the extremely high capacity of *P. hysterophorus* to translocate these metals from roots to shoots. We could not find a significant correlation between TFs of *P. hysterophorus* for Pb and Cd. But generally, the TF of *P. hysterophorus* for Cd was greater than Pb. Sorghum and alfalfa have been reported to extract the heavy metal, and transfer them to the upper part and the value of translocation factor increases as the samples as treated with EDTA (62).

Morphological attributes

The fresh and dry weight of shoots and roots decreased significantly ($P < 0.001$) after the application of treatments in both sets (Figures 5A,B and Tables 2, 3). In set-1, a minimal difference in fresh weight of shoots and roots was found in T2 when compared with the control (Figures 5C,D). Shoot and root lengths of *P. hysterophorous* were also found to be affected, minimum and maximum reductions in shoot lengths of set-1 and set-2 were found in T2, respectively (Figures 5E,F). However, in set-2, T3 had the greatest reduction in root length, followed by T2. The height of the plant got decreased and showed a stunted appearance. The lowest number of leaves and maximum leaf area of the plant were seen in T4 compared to all the treatments other than the control in both sets

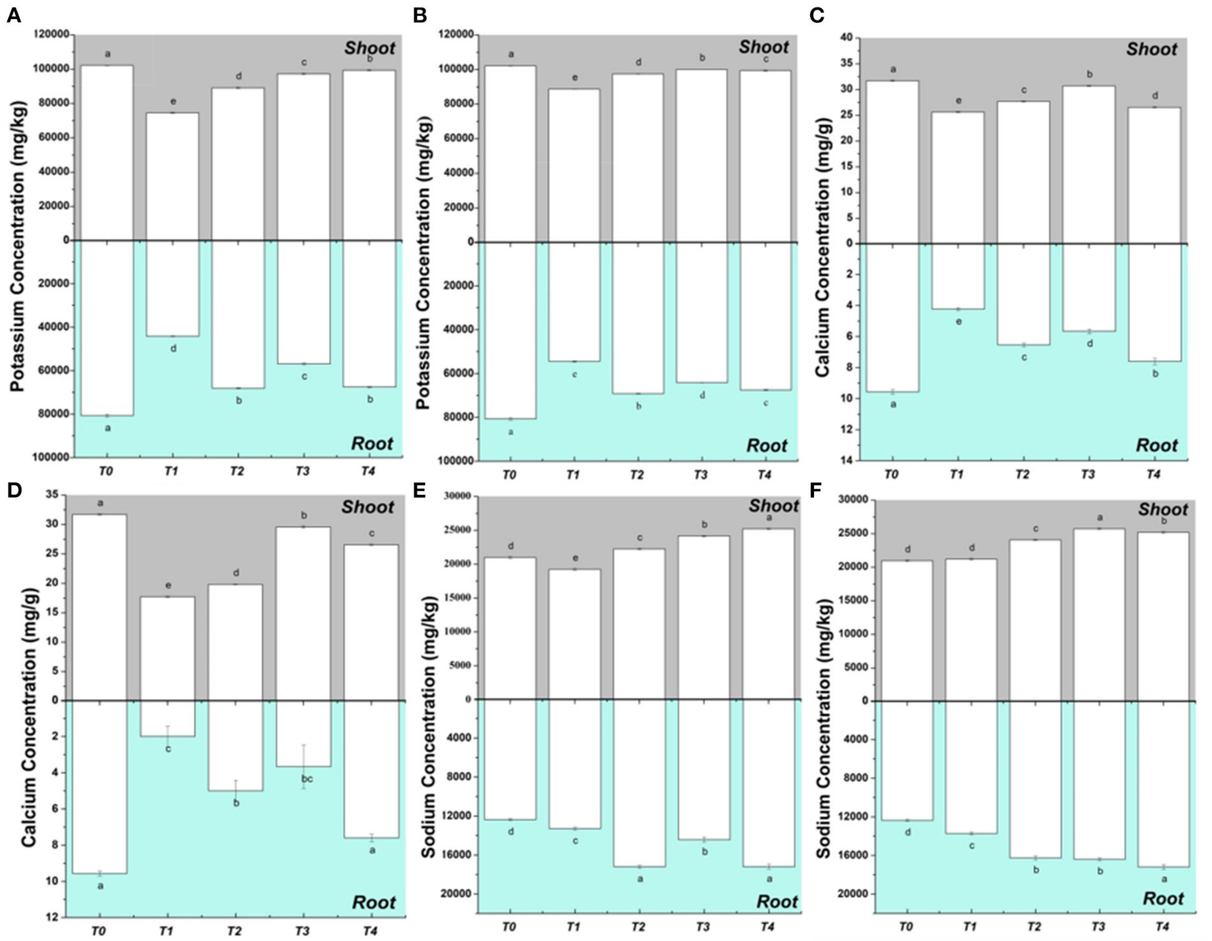

FIGURE 3
Mineral ions (K, Ca, and Na) contents in *P. hysterophorus* under the effect of various treatments in two sets. **(A)** K concentration in *P. hysterophorus* in set-1, **(B)** K concentration in *P. hysterophorus* in set-2, **(C)** Ca concentration in *P. hysterophorus* in set-1, **(D)** Ca concentration in *P. hysterophorus* in set-2, **(E)** Na concentration in *P. hysterophorus* in set-1, and **(F)** Na concentration in *P. hysterophorus* in set-2.

(Figures 5G,H). EDTA seemed to have a positive impact on shoot/root fresh and dry weights in T3 and T4 compared to T2 in set-2 in accordance with the results obtained by Kanwal et al. (63). Sudan grass and sweet sorghum grow and produce more biomass when EDTA is used as a treatment in soil (64, 65). The (EDTA + Cd) combined treatments applied to *P.hysterophorous* have significantly increased plant growth and biomass, these results also showed a positive correlation with Hayat et al. (66). EDTA treatment boosted plant growth, yield, chlorophyll content, gas exchange properties and photosynthetic parameters, by increasing antioxidant enzyme activity, and it also increased metal absorption in *Brassica napus*. (67). Another study also showed that sorghum plants grew more quickly and absorbed more nutrients when treated with metal-EDTA chelate (68). Chen et al. (69) also reported higher soybean leaf mass in the presence of EDTA under Cd stress condition.

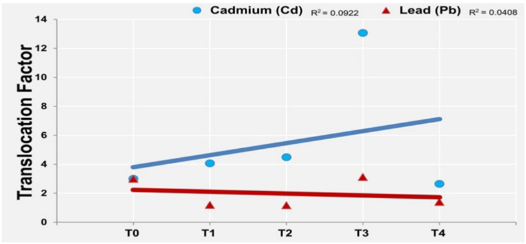

FIGURE 4
Linear correlation between translocation factors of Pb and Cd in *P. hysterophorus*.

Past research has also reported that plants exposed to Pb and Cd toxins have reduced root and shoot growth, as well as lower weight (11, 12). The increasing amount of Pb in plants is shown

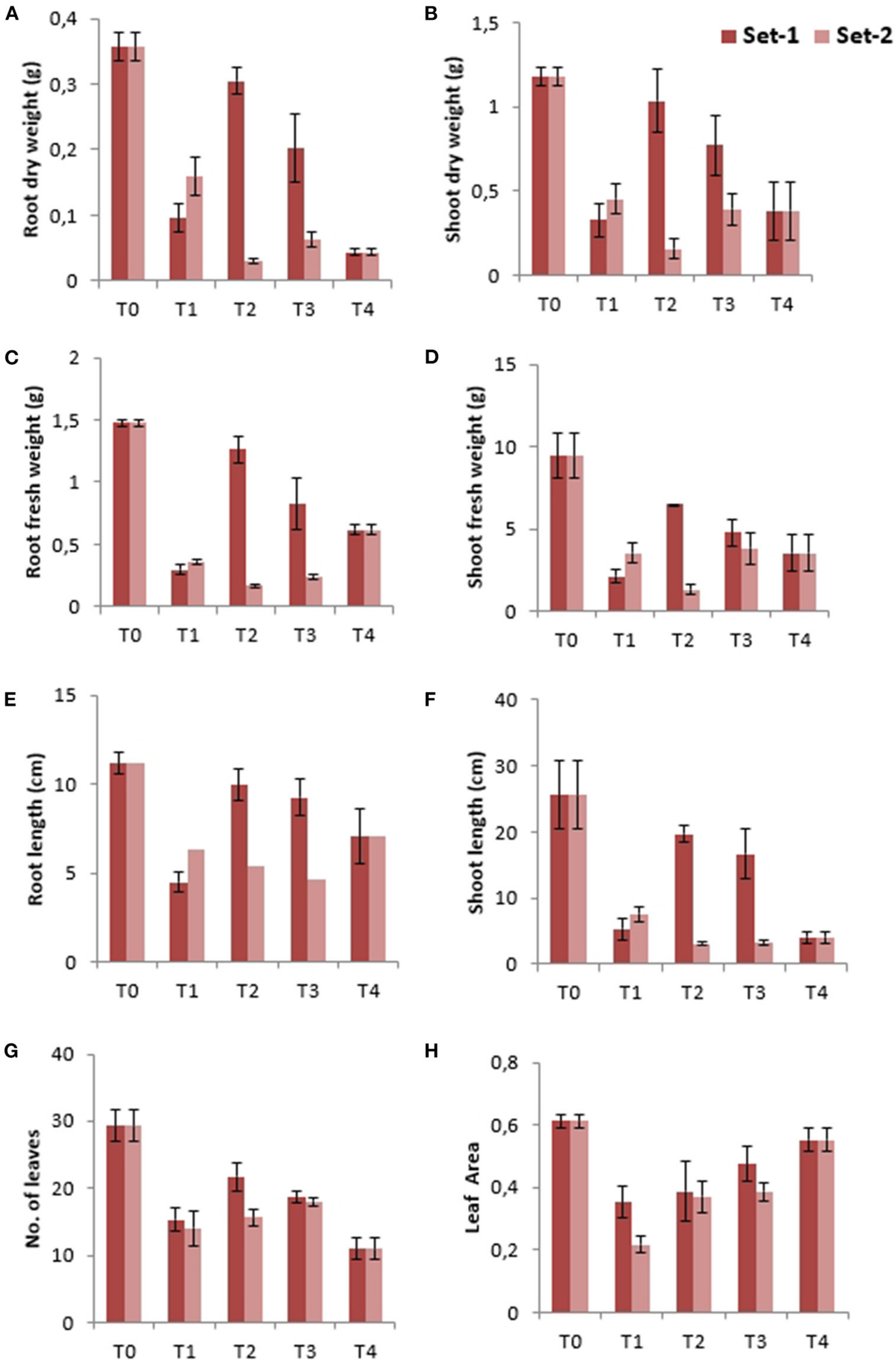

FIGURE 5

Morphological attributes of *P. hysterophorus* in two sets of treatments. **(A)** root dry weight, **(B)** shoot dry weight, **(C)** root fresh weight, **(D)** shoot fresh weight, **(E)** root length, **(F)** shoot length, **(G)** number of leaves, and **(H)** leaf area.

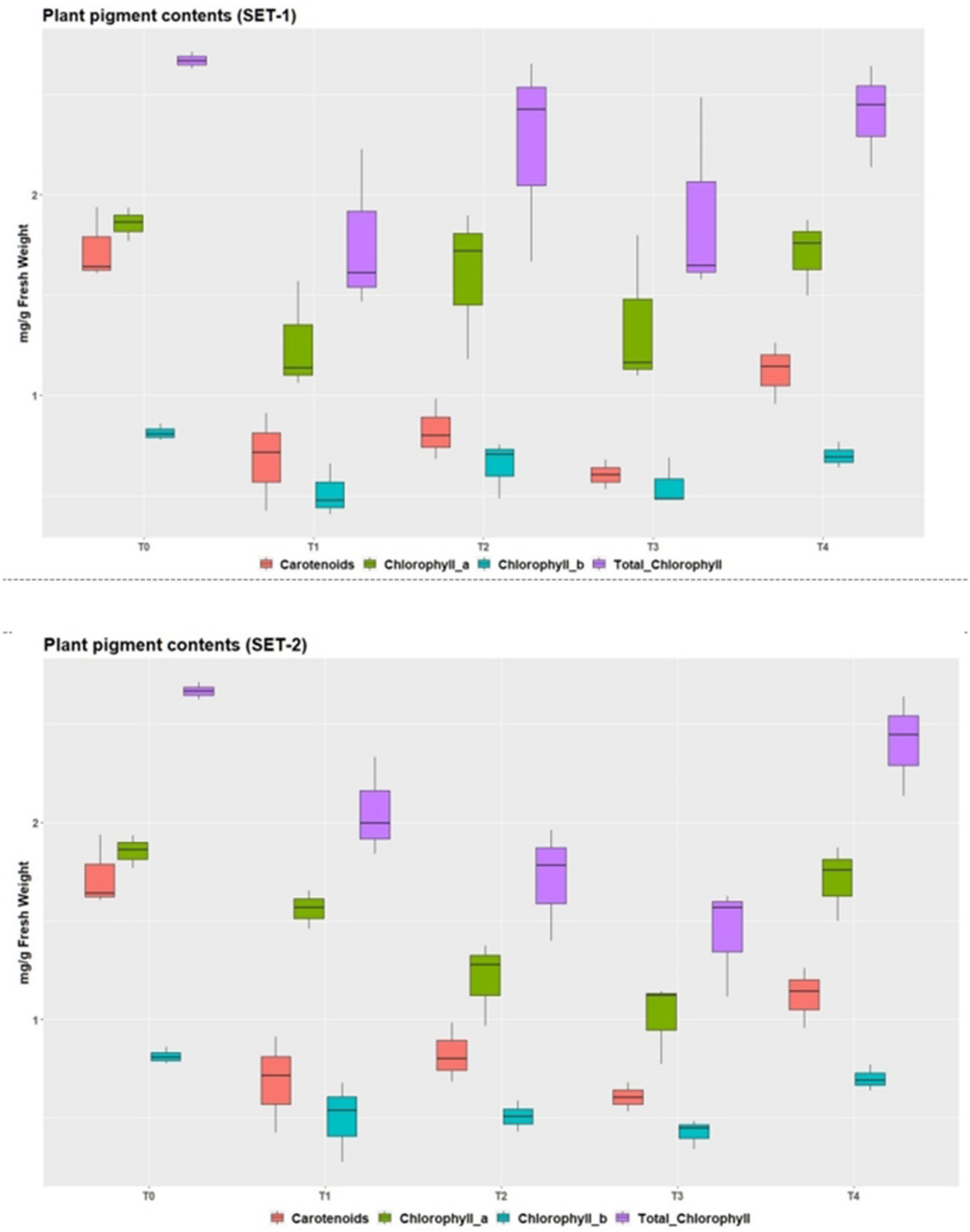

FIGURE 6
Chlorophyll pigments in *P. hysterophorus* under the effect of various treatments in two sets.

to cause visual phytotoxic effects on plants such as chlorosis and necrosis, which results in a reduced number of, leaves (70). Reduced root weight of soybean and dry biomass in Parthenium were noted as a result of Cd toxicity (44, 69). Reduced plant growth is a common symptom of metal toxicity. Heavy metal uptake happens in plants through the ion transporters in cell membranes that are meant to transport nutrient ions thereby restricting the normal metabolic activities of the plants and hampering their growth (59, 71).

Physiological attributes

Chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoids all decreased as metal intake increased. The effect of different treatments on chlorophyll contents is highly significant in both sets (Tables 2, 3). Chlorophyll a and b showed a decreasing trend from T1 to T3, but then their increase in T4 showed a positive impact EDTA had in this treatment along with metal in both sets (Figure 6). Similarly, carotenoids also showed the same trend in both sets. Similar results were obtained by other researchers as well (63, 72–75). Results are also in accordance with the Hayat et al. (66) stated that EDTA treatment raised chlorophyll content and improved plant physiology. EDTA in plants considerably enhanced plant chlorophyll content and gas exchange properties (67). The application of EDTA considerably increased the levels of chlorophyll a, b, total chlorophyll, and carotenoid content in the leaves of *B. Napus* L (63).

Chlorophyll contents are the most important biomolecules in plant cells as photosynthesis and productivity depend upon their concentration (76) noticed the direct and significant effect of heavy metals uptake on proline synthesis while a 4% reduction in chlorophyll content was seen in all the resistant/indicator species of heavy metal polluted regions. Heavy metals reduce chlorophyll contents in plants in the vicinity of leather industries of Sialkot, Pakistan (1). Various studies have shown that Cd and Pb have deleterious effects on plant chlorophyll concentration which ultimately lead to reduced photosynthetic rate and strength of the plants (5, 61, 77–79).

Conclusions

It is concluded that the Cd and Pb concentrations in shoots and roots of *P. hysterophorus* were substantially high. The capacity of *P. hysterophorus* to accumulate Pb and Cd was shown to be increased with the addition of EDTA to the soil. Morphological attributes of *P. hysterophorus* such as shoot/root length, fresh and dry weights, leaf count, and leaf area were adversely impacted by the toxic effects of Pb and Cd, however, EDTA was found to be having a positive impact by helping *P. hysterophorus* in overcoming the negative effects of heavy metals. Similarly, the same trend was recorded for photosynthetic pigments. Ions i.e. K and Ca got reduced in Pb and Cd treatments, But EDTA in T3 and T4 helped in restoring the amount of these ions in *P. hysterophorus*. Na concentration, however, in all of the treatments except the control, was found to be significantly higher. We conclude that *P. hysterophorus* can uptake and accumulate high concentrations of Cd and Pb and this ability can be enhanced by the application of EDTA in the soil. We recommend using this highly proliferating plant species for remediation of Pb and Cd contaminated soils, but further research is required in this regard in the natural environment and for some other metal types. Decreasing the toxic level of heavy metals in the soil in accordance to the WHO standards can improve the ecosystem as well as general public health that is the prime objectives of scientists.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

UE: conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, and roles/writing—original draft. SK: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, software, supervision, validation, visualization, and writing—review and editing. MA: software, supervision, validation, visualization, and writing—review and editing. NK: supervision, methodology, resources, validation, visualization, and roles/writing—editing original draft. WS: formal analysis, investigation, methodology, software, validation, and visualization. NN: formal analysis, investigation, methodology, validation, and visualization. AR: visualization, supervision, project administration, funding acquisition, and writing—review and editing. HH, JY, and GY: project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Responding to the need of postgraduate education for Planetary Health: Development of an online Master's Degree

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Planetary Health has emerged as a new approach to respond to the existential risks that the clime and global environmental crises pose to human societies. As stated by various stakeholders, the challenges involved in Planetary Health are of such magnitude that education must be at the forefront to obtain a meaningful response. Universities and higher education institutions have been specifically called to embed the concept of planetary stewardship in all curricula and train the next generation of researchers and change makers as a matter of urgency. As a response to this call, the Universitat Oberta de Catalunya (UOC), the Universitat Pompeu Fabra (UPF), and the Barcelona Institute for Global Health (ISGlobal) developed the first online and asynchronous Master in Science (MSc) in Planetary Health. The aim of the programme is to train a new generation of academics and professionals who understand the challenges of Planetary Health and have tools to tackle them. This article describes the development of the curriculum of this MSc, presents the main characteristics of the programme and discusses some of the challenges encountered in the development of the programme and its implementation. The design of this MSc was based on: the alignment of the programme with the principles for Planetary Health education with a focus on human health; a multi-, inter-, and trans-disciplinary approach; the urgency to respond to the Anthropocene challenges; and the commitment to the 2030 Agenda. The MSc was recognized as an official degree by the Agency for Quality of the Catalan University System, included in the European Quality Assurance Register for Higher Education, and the Spanish National Academic Coordination body in April 2021 and launched in October 2021. There are currently more than 50 students enrolled in the program coming from a broad range of disciplines and geographic locations. The information presented in this article and the discussion on challenges encountered in developing and implementing the programme can be useful for those working in the development of similar programs.

KEYWORDS

curriculum development, education for sustainable development (EfSD), Master in Science, planetary health education, postgraduate education, sustainable development goals –SDG

Introduction

Background and rationale for the educational activity innovation

In the last decades, there has been an increasing understanding of the socio-environmental transformations—accelerated by the Anthropocene —and how they pose an existential risk to human societies and other living beings. As a result, several approaches emerged to connect the environment and human health (1, 2) and respond to new threats, such as the climate crisis, biodiversity loss and toxic pollution (1). One of the new approaches, Planetary Health, is based on the comprehension that human health and human civilization depend on flourishing natural systems and their stewardship. This point of view requires unprecedented efforts to deal with complexity and uncertainty, encourage transdisciplinary and urgent action (3).

Similar to other scientific fields (4), the challenges involved in Planetary Health are of such magnitude that education at all levels must be at the forefront to obtain a meaningful response (5, 6). The São Paulo Declaration on Planetary Health, a global call to action from the planetary health community supported by more than 300 signatories, urged to include planetary health education in all curricula levels, from schools to universities (7). The UN report "The Future is Now" has specifically called upon universities and higher education institutions to support the mission of advancing sustainability. This recognizes that the education of the next generation of researchers and change makers is one of the best leverage strategies toward transformations in sustainability (8). Recently, the 'Our Planet, Our Future" call for action—signed by a large number of Nobel laureates—requested universities and higher education institutions to urgently embed the concept of planetary stewardship in all curricula (9).

In consequence, there is a growing number of initiatives to transform higher education for sustainable health. The Association of Medical Education in Europe (AMEE) has suggested that to reduce CO_2 emissions and to meet the UN's 2030 Sustainable Development Goals (SDGs), health-related studies must equip undergraduates (and already qualified professionals) with the necessary knowledge, skills, values, competence, and confidence (10). The Global Consortium on Climate and Health Education (GCCHE) surveyed 160 institutions to understand the state of climate-health curricula for health professions. The results showed that educational programmes vary considerably between institutions and that the majority of responders faced relevant challenges when trying to implement curricular changes in their institutions (11). A similar study conducted in Latin-America has shown that universities in this continent have similar drivers and barriers for sustainability change as universities in other geographical contexts (12).

There is growing evidence of new methodologies and approaches to include Planetary Health in health curricula (13–15). Among the different types of curricula, postgraduate education has received little attention and yet it offers a unique opportunity to train already qualified professionals from different disciplines to work multi- (drawing on knowledge from different disciplines but remaining within the boundaries of those fields), inter- (analyzing, synthesizing, and harmonizing links between disciplines into a coordinated and coherent whole), and trans-disciplinary (using a shared conceptual framework drawing together new disciplinary-specific theories, concepts, and approaches to address common problems) (16). In the GCCHE study cited above, only one institution reported having a master's or certificate programme in climate and health. Its respondents reported that it had been virtually impossible to develop new courses on climate and health in public health master's programmes due to the already high course load (11). To contribute to the development of Planetary Health education at the postgraduate level, the *Universitat Oberta de Catalunya (UOC)*, the *Universitat Pompeu Fabra (UPF)*, and the Barcelona Institute for Global Health (ISGlobal) have developed an online Master in Science (MSc) in Planetary Health. It was launched in October 2021 and will be fully implemented in March 2023.

This article describes the main characteristics of this new programme and discusses some of the challenges we are currently facing.

Overview of the master in science in planetary health

The MSc in Planetary Health (UOC-UPF-ISGLOBAL) is a fully online and asynchronous programme of 60 ECTS credits (European Credit Transfer and Accumulation System, 1 ECTS is equivalent to 25 h). In its first edition, the MSc in Planetary Health has been offered in Spanish and Catalan. The academic entry requirements encompass undergraduate studies from a broad range of disciplines, including health sciences, natural sciences, political sciences, economical sciences, sociology, law, and engineering. The first cohort of students (first term October 2021) captures this multidisciplinary profile (see Figure 1). This fist cohort was integrated by 55 students (75% females, 25% males), 87% of them were from Spain, 7% from other European countries and 5 % from Latin America.

The overall aim of the programme is to provide a multi-, inter- and transdisciplinary academic syllabus, as well as applied education on Planetary Health to train, promote, and empower a new generation of academics and professionals. They will be able to contribute to understanding the full complexity of the challenges related to Planetary Health and well-being; from which they will develop and find solutions and strategies to tackle these challenges. To achieve this aim, the design of this MSc degree was based on a set of general criteria: (i) the programme content is aligned with the principles for Planetary Health education (17) with a focus on human health; (ii) it includes the essential multi-, inter-, and trans-disciplinary aspects of Planetary Health challenges; (iii) it transmits a sense of urgency as a consistent attitude, considering the timeframe of the challenges that involve climate and the Earth's natural systems; and (iv) it is aimed to create an impact and it is committed to the 2030 Agenda.

This MSc programme was recognized as an official degree by the Spanish academic system on April 2021. Official MSc allow the enrolment in PhD programs and therefore are a way to promote research in a given field. Official degrees are subjected to a thorough evaluation process: this programme was evaluated and approved by the Agency for Quality of the Catalan University System (AQU Catalunya), included in the European Quality Assurance Register for Higher Education (EQAR), and the Spanish National Academic Coordination body (*Consejo de Universidades*). The programme was also reviewed and supported by an international advisory committee, which involved researches and academics working in areas relevant to Planetary Health.

Methods

Definition of the programme

The definition of the programme was a collaborative process in which several actors were involved. Throughout several group discussions, the direction of the programme and the academic committee (https://estudios.uoc.edu/es/masters-universitarios/salud-planetaria/profesorado) agreed on the structure and the contents of the programme (presented in the following section). The academic committee included scholars working in multiple disciplines, such as public health, environmental epidemiology, climate sciences, ecology, political ecology and political economy. Specialist in pedagogy and educational methods from the eLearning Innovation Center (eLinC) centre at UOC (https://www.uoc.edu/portal/en/elearning-innovation-center/coneix/index.html), contributed in the definition of the specific learning outcomes and identify the best methodologies to achieve them. Figure 2 describes the steps followed to prepare

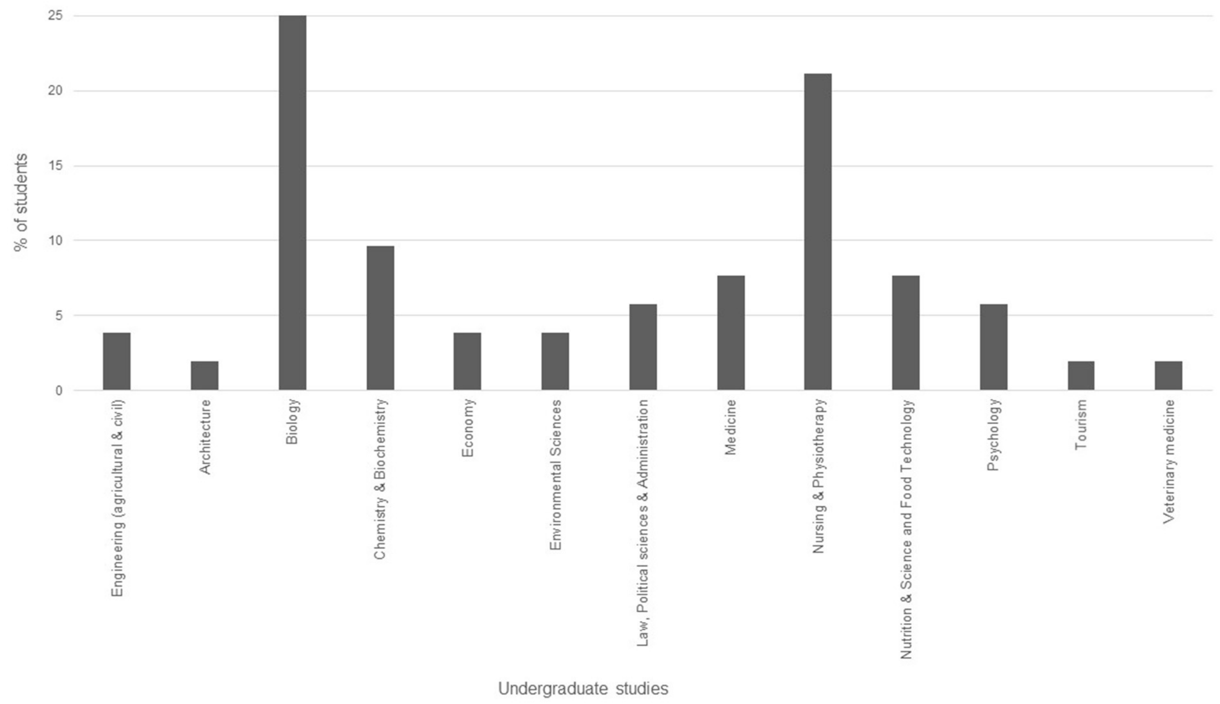

FIGURE 1
Description of the academic backgrounds of the first cohort of the MSc in Planetary Health (*n* = 55).

the final version the programme and the actors involved in each step of the process.

Structure and coherence of the programme

The MSc in Planetary Health (UOC-UPF-ISGLOBAL) is organized in twelve modules of 5 ECTS each (equivalent to 125 h) and structured in three thematic blocks (see Table 1). The first block (three modules) provides the general context and the necessary methodologies for understanding and responding to the Planetary Health challenges of the Anthropocene. It also sets the bases for an effective multilevel global governance. The second block (which includes six modules) focuses on issues identified as key challenges for Planetary Health: food systems, change in land use and loss of biodiversity, water resources, global pollution, urbanization, healthy and sustainable cities, and the climate emergency. The main objective is to develop the student's critical understanding of the origins and causes of these issues and its effects on human health to devise and design potential solutions, as well as to evaluate possible problems and risks when implementing them. In that sense, all modules from block 2 are solution oriented and are designed according to this scheme: (i) description of the challenge, (ii) potential solutions to the challenge, and (iii) evaluation of the possible problems and risks when implementing them. The third block (which includes three modules) integrates and applies the concepts from the two previous blocks. It includes a module with strategies that promote transformative changes to address the challenges of Planetary Health, including the role of citizen action and social movements; a second module to introduce research on Planetary Health and familiarize students with a broad range of research areas and disciplinary approaches; and a third module, focused on the master's thesis. The specific competences and their distribution thought the modules are presented in Table 2. Further details on each module can be found in the Supplementary material (Supplementary material: Curriculum of the MSc in Planetary Health).

To make the MSc in Planetary Health a coherent programme, its structure and contents were aligned with the Planetary Health educational framework and the SDGs. Moreover, the competences and learning outcomes (see Table 1) cover the majority of overarching principles for Planetary Health education (17).

The MSc encompasses the five domains (see Table 1) proposed by the Planetary Health educational framework (18). The domains on "*The Anthropocene and Health*, "*Systems Thinking and Complexity*" and "*Equity and Social Justice*" are mainly addressed on the first thematic block of the programme.

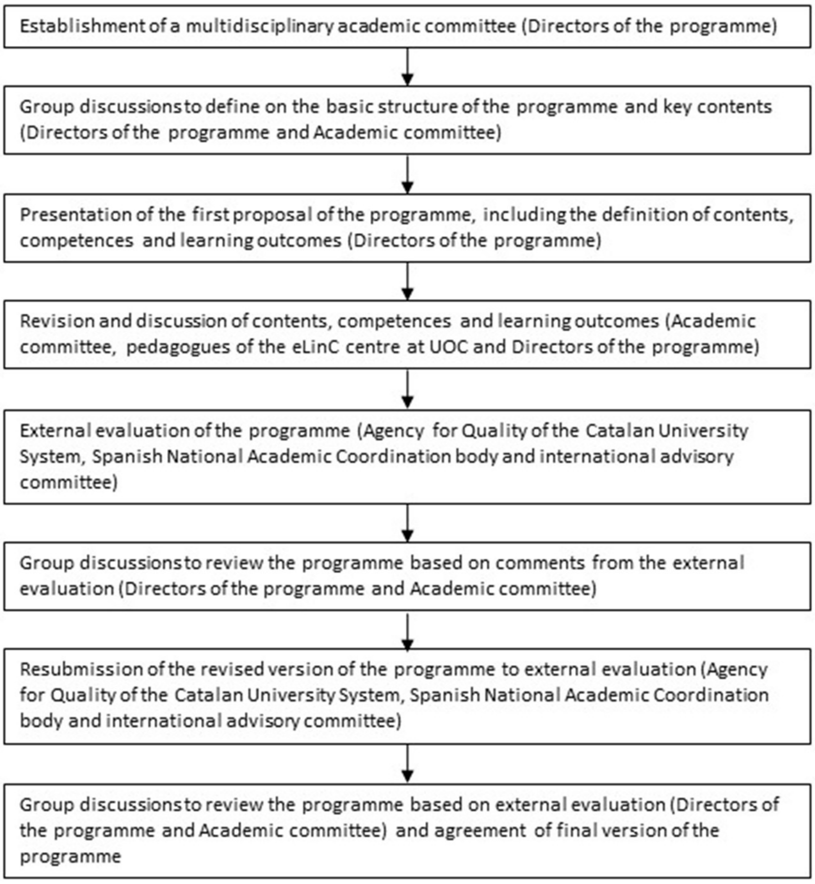

FIGURE 2
Steps followed to define the programme and the actors involved in each step.

The need to understand human beings and natural systems as interconnected entities ("*Understanding Interconnection within Nature*" domain) is a cross-cutting theme in all subjects of the degree, and it is especially relevant in subjects of the second thematic block. In this second block, students have to reflect on the root causes of the global environmental crisis, leading them to recognize our disconnection from nature. The domain on "*Movement Building and Systems Change*" is mainly covered in the third thematic block of the master; it is oriented towards integrative solutions and transformative changes. In addition, the five domains are—up to certain level—incorporated in all modules, providing additional coherence to each module and throughout the programme.

The SDGs framework is embedded in the conceptualization of the programme. It is present in the different learning and teaching materials, as well as in the activities. For example, in the module "Planetary Health, the Response to Anthropocene Challenges," students are asked to explore how SDGs could operationalize the concept of Planetary Health. This exercise aims to clarify that sustained improvements in human health and well-being are linked to the preservation of key natural systems, and supported by good governance and appropriate policies (3). However, the 2030 Agenda is also approached from a critical point of view through the programme discussing the need of a more ambitious and urgent framework to deal with the climate and ecological crises and acknowledging some of the criticisms the Agenda received (19). The module "Global and multilevel governance in Planetary Health" integrates this critical vision. by shedding light on the difficulties and governance challenges associated with the implementation of the most relevant SDGs for Planetary Health.

Learning environment and pedagogical format

The degree has been implemented following the UOC asynchronous online educational model (20), which is

TABLE 1 Organization of the MSc in Planetary Health in relation to the domains defined in the Planetary Health educational framework (18).

Tematic blocks	Modules	Main domain covered
Block 1: concepts and methods	1. Planetary Health, the response to the challenges of the Anthropocene	The anthropocene and health
	2. Interdisciplinary approaches to Planetary Health	Systems thinking and complexity
	3. Global and multilevel governance in planetary health	Equity and social justice
Block 2: challenges in planetary health	4. Sustainable food systems and healthy diets	Understanding interconnection within nature
	5. Land use change, biodiversity loss and human health	
	6. Water resources and Planetary Health	
	7. Global pollution and health	
	8. Urbanization and healthy and sustainable cities	
	9. Climate change and health	
Block 3: application of knowledge	10. Integrative solutions and transformative changes	Movement building and systems change
	11. Planetary Health Research: From the hypotheses to the research protocol	
	12. Master's thesis	

underpinned by two principles: learning by doing and autonomous learning. Following this model, each module of the MSc in Planetary Health is organized around the resolution (or response) to a number of challenges. The challenges are inspired by real contexts of the different disciplinary areas and are oriented to develop defined personal and professional skills (see Supplementary material). This is achieved by asking the student to complete a series of activities and/or prepare deliverables for each of the challenges. The evaluation of the modules is based on the continuous assessment of such activities and deliverables during the term.

Each of the challenges includes: the approach to the addressed issue, the description of activities to develop key skills, and the learning resources and tools to complete the activities and/or prepare the deliverables. Learning resources are found in the virtual classroom in a visual way. For each resource, guidance on how to use it in the context of the activity and the expected amount of time required to complete each challenge is provided. The learning resources available have a wide range of formats (websites, video, audio, texts, or digital tools); and include both teaching resources prepared by faculty members and external resources (academic papers, book chapters, scientific reports, recorded academic conference presentations, infographics, and documentaries, among others).

During the autonomous learning process, students are supported by the faculty. In the UOC educational model, there are two faculty roles: coordinating professors and course instructors. Coordinating professors design the content of the module, coordinate and supervise the team of course instructors, and supervise the evaluation process. Course instructors are in close contact with the students by introducing the activities for each challenge, promoting participation in the virtual classroom, solving specific questions, providing feedback, and evaluating the activities and deliverables.

The multi-, inter-, and trans-disciplinary aspects of the programme are central to Planetary Health (3) and a hallmark of the MSc in Planetary Health. The syllabus has been developed by a multidisciplinary academic committee, including experts on public health, environmental epidemiology, climate change, political ecology and geography, international law, social sciences, economics, and ecology. All modules are regularly reviewed and discussed by the academic committee, ensuring that diverse approaches and views are adequately considered.

In the Supplementary material, we present an example of how the UOC educational model is applied to one of the modules of the MSc in Planetary Health (Supplementary material: Example of the implementation of UOC educational model in the MSc: Module 1 Planetary Health: The Response to Anthropocene Challenges).

Anticipated career trajectories

The programme was planned to promote a new generation of professionals and academics able to apply a Planetary Health approach in their careers. Specifically, the programme aims to provide capabilities in research, education and professional work.

As official degree by the Spanish academic system, our programme incorporates a strong focus on research as the source of new knowledge. All modules strengthen the importance of knowledge generation as part of the solution to the climate and environmental crises. Also, the programme provides a research path for those interested in pursuing a PhD programme. Thus, following a career as researcher is one of the possible trajectories for our graduates.

Graduates are also anticipated to develop careers in education and several of our registered MSc students are already teaching in high-schools and universities. As commented above,

TABLE 2 Distribution of competences and skills across the modules of the programme.

Competences and skills	Modules											
	1	2	3	4	5	6	7	8	9	10	11	12
Basic competences and skills												
CB6 – Gain and understand knowledge that forms the basis or an opportunity for original thinking in the development and/or application of ideas, typically in a research context	x		x								x	x
CB7 – Capacity to apply the acquired knowledge and capacity for problem-solving in new or relatively unknown environments within broader (or multidisciplinary) contexts related to the field of studies.			x		x	x						x
CB8 – Capacity to integrate knowledge and tackle the complexity of formulating judgements based on incomplete or limited information, taking due consideration of the social and ethical responsibilities involved in applying knowledge and making judgements	x			x	x	x	x	x	x			x
CB9 – Capacity to communicate conclusions and the knowledge and grounds on which they have been reached to specialist and non-specialist audiences in a clear and unambiguous manner				x				x				x
CB10 – Learning skills that enable ongoing self-directed and independent learning				x	x	x	x	x				
General competences and skills												
CG1 – Capacity to search for, analyze, assess, and use information provided to make decisions in complex situations	x	x			x	x	x					x
CG2 – Capacity to work in interdisciplinary teams to attain shared goals in relation to planetary health		x			x		x			x		
CG3 – Capacity to apply creative thinking to contribute improvements or solutions in areas and situations of varied complexity in relation to planetary health			x						x	x		x
CG4 – Capacity to resolve complex situations in a feasible, sustainable way, by analyzing their components, finding alternatives, reaching consensus on their application and assessing the results of their implementation												
Transversal competences and skills												
CT1 – Capacity to act in an honest, ethical, sustainable, socially responsible and respectful way considering human rights and diversity, both in academic and professional practice, and design solutions to improve these practices		x	x							x	x	x
Specific competences and skills												
CE1 – Analyze the interaction between human health and the Earth's natural systems, using complex theoretical and conceptual models that relate the factors that promote environmental change, their effects on health, and allow for possible solutions to guarantee health in a sustainable way	x			x	x	x	x	x	x	x		
CE2 – Design research projects and interventions, applying and integrating advanced knowledge on socioeconomic, political and / or cultural factors that interact affecting human health and promoting the degradation of natural systems				x	x			x			x	x
CE3 – Critically interpret, synthesize and integrate the results of quantitative and qualitative analysis from research in the main scientific disciplines related to Planetary Health (health sciences, natural and climate sciences, social sciences and economics)		x					x	x			x	x
CE4 – Select and apply advanced methodologies and resources from different scientific disciplines in the field of Planetary Health to strategically solve complex situations and problems	x										x	x

(Continued)

TABLE 2 (Continued)

Competences and skills	Modules											
	1	2	3	4	5	6	7	8	9	10	11	12
CE5 – Mastering the language and communicative conventions of the disciplinary fields of Planetary Health in order to act as an interlocutor in the professional field, formulating arguments and transmitting results and ideas in a clear and unambiguous way		x	x					x	x			x
CE6 – Implement with initiative and autonomy research projects or interventions in the field of Planetary Health, integrating a multidisciplinary vision, transferring the main results to the actors involved											x	x
CE7 – Critically evaluate and apply innovative proposals for solutions for the prevention, promotion and management of health with a multidisciplinary approach, taking into account environmental sustainability and equity				x	x	x	x	x	x	x	x	x
CE8 – Formulate predictions about the evolution of health problems based on changes in natural systems, through innovative and multidisciplinary approaches that consider socioeconomic, political and / or cultural factors	x	x	x	x					x			x

inclusion of the Planetary Heath in the curricula of all programs and faculties has been also stated as a priority by various stakeholders (5–9). Though our MSc does not provide training in education methods, it is proving useful both for those in education to incorporate planetary health contents in their programs as well as to acquire training in the adoption of online education methods.

Finally, and consistent with the consequential and solution-oriented spirit of planetary health, our MSc is also strongly oriented to support career opportunities for professional working in public and private sectors directly dedicated to health and the management of natural resources, urbanization, or transport among others. For instance in (i) international agencies and / or non-governmental organizations (NGOs) that work in the field of health, environmental preservation and/or sustainable development, (ii) health services that seek to integrate aspects of environmental sustainability in health care and management, (iii) public administration that works in the development and / or implementation of land management plans, town planning or energy, among others or (iv) in the sustainability and corporate social responsibility departments of companies related to health and the environment.

Discussion

This article describes the development of a multidisciplinary online master degree on Planetary Health, showing its structural consistency with the Planetary Health educational framework (18) and the UN's Agenda 2030. In concordance with the urgency that the climate and ecological crises request (21), the new degree has received the support of the national accreditation bodies and has attracted a first cohort of students with a wide range of academic backgrounds in a very short period of time. We have integrated the guiding criteria of complexity, multidisciplinary, and urgency (3) to develop a feasible and innovative programme for postgraduate education in Planetary Health, which is also consistent with a wider set of cross-cutting principles for Planetary Health education (17).

Planetary Health involves a paradigm shift compared to global public health. Planetary Health integrates human health with the health of other species and Earth's natural systems, something that implies a broader ethical perspective to explicitly account for the value of future human and other species generations. Planetary Health also extends the global public health predicament of social sciences approach in dealing with health inequalities and equity to the understanding of the interactions between social systems and ecosystems and to deliver solutions to protect and restore the natural systems on which human health depends. Planetary Health shares this paradigm shift with other approaches. Busse et al. (1) have analysed some of the health approaches that connect the health of ecosystems, other living organisms and humans, including occupational and environmental health; political ecology of health; environmental justice; eco-health; One Health; and ecological public health. The proliferation of approaches emerging from different disciplinary fields, can lead to confusion due to overlaps in concepts and terminology. As a result of the new approaches, there are numerous initiatives to align postgraduate education in the health sector with the challenges of the climate crisis and the Anthropocene. For instance, the Faculty of Public Health and Training Programme Directors—from the 13 public schools specialized in health across the UK—have recently reported an initiative to strengthen sustainable development in public health consultant education (22). Postgraduate education in One Health has already come a long way with a large number of master programmes, which either highlight One Health in the programme's name or include it as a feature component (23). In contrast, postgraduate education initiatives focusing on Planetary Health are less common. To our knowledge there are no other master degrees on Planetary Health. However, we are aware of other universities planning to launch masters on Planetary Health soon and the Stanford University and the London School of Hygiene & Tropical Medicine (LSHTM) have recently launched a Planetary Health Postdoctoral Fellowship programme (24). Moreover, It is very likely that many schools of public health are currently developing specific postgraduate modules on Planetary Health like the ones in the University of Toronto Dalla Lana School of Public Health (25) and the UPF (26).

Beyond the described initiatives, to our knowledge, our programme is one of the first MSc degrees fully devoted to Planetary Health. A relevant innovative component is its online and asynchronous methodology, responding to a call by young academics to expand online training for Planetary Health, which can contribute to boost access to high-quality education programmes for global audiences (5) and foster education at different stages of the professional career. The MSc is available in Spanish, offering an opportunity to expand Planetary Health education to Spanish-speaking countries. English version is currently being considered.

A major consequence of the global and complex nature of Planetary Health challenges is the need of close collaborations across different disciplines; the approaches towards such collaboration are diverse and they can be multi-, inter-, and trans-disciplinary. The need to adopt these approaches is based on the assumption that the Anthropocene's challenges are so complex that their thorough examination and solution requires different scientific disciplines to work in alliance with each other, something that requires the combination of efforts and knowledge from different disciplines, including but not limited to medicine, biology, climate science, economics, political science, law, humanities, culture or technology (27). To achieve this in our programme, we have drawn from our own experience with the Planetary Well-being Initiative, (15) as well as from previous calls for a cross-disciplinary collaboration in Planetary Health (3, 18, 20).

Postgraduate education provides a unique opportunity for students with different disciplinary backgrounds to meet in inter-professional education programmes (28). The importance of multidisciplinary and inter-professional education has been largely recognized in the postgraduate education of public health and global health (28, 29) and we have built on this tradition.

The multi-, inter-, and trans-disciplinary approach in the educational programme allows students to share a common set of conceptual models and to be exposed to a wide range of methods. This will strengthen their capacity to work alongside colleagues from other disciplines throughout their careers. The adoption of a multi-, inter-, and trans-disciplinary approach (16) has several implications, such as the need of a faculty with a diverse background in the disciplines involved in the *corpus* of Planetary Health. In the current stage of the MSc in Planetary Health, the diversity of disciplinary backgrounds is well-stablished in the team of course instructors, in the academic committee that supervised the design of the programme and the modules, and in the external advisory committee who played a key role during the accreditation phase. However, the adoption of this approach also involves some relevant challenges. One of them is to provide students with additional contents to be able to follow those topics that are far from their own academic background. Another very important challenge is to guarantee that all students receive an advanced education level with a strong focus on research (instead of an introductory education to a wide subject's thematic range). So, adopting a wide multi-, inter-, and transdisciplinary approach in a master degree involves a tension between the large diversity of theories, concepts and methods and the degree of deepness that a master level requires. This tension was already noted by the evaluators in the accreditation phase. To address these difficult issues, we are conducting targeted actions that require close monitoring and evaluation of the MSc programme.

A potential limitation of the MSc at its current stage is the limited presence of local knowledge (e.g., traditional indigenous knowledge and local ecological knowledge) in the curricula. The inclusion of local knowledge and ideas to navigate sustainability locally is in general poorly addressed within higher education (30). However, we believe that including this content in the curricula will emphasize the importance of the human-nature relationship in other worldviews, as well as recognize the relevance of traditional knowledge in monitoring changes in nature and in providing examples of successful adaptation to these changes (31–33). To address this issue, we will explore collaborations with indigenous scholars to further integrate local knowledge in the MSc.

Universities and higher education institutions have a pivotal role in Planetary Health: (3) they have been called to urgently embed the concept of planetary stewardship in all curricula (9). In our strategy to include Planetary Health in the university, we have maintained a sense of urgency as a core principle (15). However, dealing with the complexity of university bureaucracy and complying with the necessary academic accreditation standards requires time and resources. For us, it was key to have a strong support from the participating institutions. This allowed us to develop the programme, obtain the approval of the Spanish academic system, elaborate and implement the initial learning resources, and recruit the first cohort of students in about 2 years. We lacked the reference of any previous MSc in Planetary Health, which made our task more challenging.

The motivation letters of our students show a strong desire for educational programmes that provide them with academic training on relevant scientific knowledge and training to become planetary stewards. We hope that our experience is useful and inspiring for other institutions to create similar programmes, which would result in further opportunities for collaboration and mutual learning. Our goal is to generate a critical mass of professionals with Planetary Health knowledge and values, willing and able to coordinate inter-professional teams and to work effectively with cross-sector stakeholders to solve today's and tomorrow's pressing challenges.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical review and approval was not required for this study in accordance with the local legislation and institutional requirements. Written informed consent from the academic committee participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

JA and CO'C-G are the directors of the programme, conceptualized the curriculum, the paper, and wrote the original daft. AM contributed to the writing of the original daft and prepared the figures. MB-P, JJ, RL, CT, and CZ contributed to the conceptualized the curriculum and reviewed and edited the original draft of the article. EC-S, PD, CG, AG-J, MG, OG, HM, FM, LM, GN, AN, IR-M, NS-V, and MT-M reviewed and edited the original draft of the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Review of major meat-borne zoonotic bacterial pathogens

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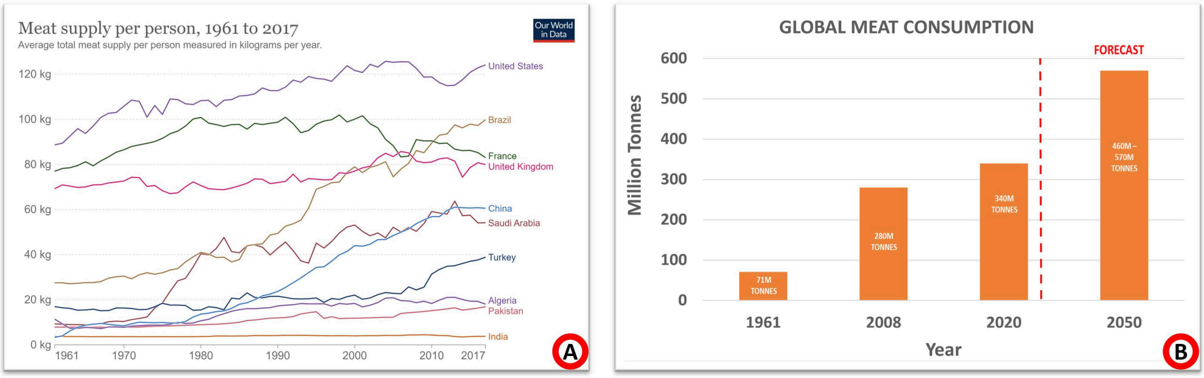
The importance of meat-borne pathogens to global disease transmission and food safety is significant for public health. These pathogens, which can cause a variety of diseases, include bacteria, viruses, fungi, and parasites. The consumption of pathogen-contaminated meat or meat products causes a variety of diseases, including gastrointestinal ailments. Humans are susceptible to several diseases caused by zoonotic bacterial pathogens transmitted through meat consumption, most of which damage the digestive system. These illnesses are widespread worldwide, with the majority of the burden borne by developing countries. Various production, processing, transportation, and food preparation stages can expose meat and meat products to bacterial infections and/or toxins. Worldwide, bacterial meat-borne diseases are caused by strains of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Shigella*, *Campylobacter*, *Brucella*, *Mycobacterium bovis*, and toxins produced by *Staphylococcus aureus*, *Clostridium* species, and *Bacillus cereus*. Additionally, consuming contaminated meat or meat products with drug-resistant bacteria is a severe public health hazard. Controlling zoonotic bacterial pathogens demands intervention at the interface between humans, animals, and their environments. This review aimed to highlight the significance of meat-borne bacterial zoonotic pathogens while adhering to the One Health approach for creating efficient control measures.

KEYWORDS

meat borne zoonoses, *Escherichia coli*, *Salmonella*, *Listeria*, *Brucella*, food intoxication, antimicrobial resistance, One Health approach

Introduction

Humans are considered omnivores because they have been eating meat for about 2.6 million years. Meat is a prominent source of protein in the average person's diet. Additionally, it has been shown that fortifying different lentils can ease the pressure on meat consumption (1). Since 1961, the amount of meat produced worldwide has more than quadrupled. Pork is the most consumed meat, although the poultry supply is expanding rapidly. Meat consumption varies widely among countries depending upon various factors, including the country's economy, culture, and more. Figure 1A depicts the amount of meat consumed by one individual in selected countries since 1961. It has been demonstrated that countries with a higher per capita income consume more meat than those with a lower per capita income. The world produces almost 340 million metric tons of meat a year, three times more than 50 years ago.



Source: UN Food and Agriculture Organization (FAO) OurWorldInData.org/meat-production • CC BY
Note: Data excludes fish and other seafood sources. Figures do not correct for waste at the household/consumption level so may not directly reflect the quantity of food finally consumed by a given individual.

FIGURE 1
Global meat consumption and production since 1961. **(A)** Summarizes a select group of nations with annual meat consumption per person. Broadly speaking, the wealthier a country is, the more it consumes meat. Most people in low-income countries still consider meat a luxury item. **(B)** Summarizes global meat production/consumption. Compared to 1961, meat production is now four times as high. By 2050, it is predicted to rise to between 460 and 570 million metric tons.

Meat consumption is estimated to rise by 460–570 million metric tons by 2050, as described in Figure 1B. It is estimated that the global consumption of meat will exceed 328 million metric tons in 2021 (2). Pathogenic diseases, such as lumpy skin disease, are becoming increasingly common in key livestock countries, which poses a major threat to the global supply of meat and meat products (3).

Among the numerous microbes interacting with animals, some of these pathogens may become zoonotic and cause illness among humans, posing a threat to public health and the economy. Animal-derived food products, including milk, meat, and eggs, are considered essential components of human nutrition (4). However, food contaminated with pathogenic microbes may pose a serious threat to public health. These diseases can be as mild as self-limiting diarrhea or as fatal as cancerous conditions. It has been estimated that contaminated food is the source of illness for 1 out of 10 people (5). Food-borne infections are more common in children under five, who account for 40% of all cases, burdening the healthcare system and impeding a nation's socioeconomic development (6).

Meat-borne diseases (MDBs)

Meat, red or white, from mammalian, avian, amphibian, aquatic, and reptilian species is consumed by humans as food. It is an excellent source of proteins, vitamins, and minerals and contains essential amino acids. Food products that are consumed raw are considered a direct source of food-borne infections. These include unpasteurized milk, raw eggs, undercooked meat, and uncooked shellfish (7). Depending on the animal's health and the hygienic conditions of the meat processing facilities, meat can be a source of many different pathogens. These pathogens can enter the food chain either by direct infection of animals or by contamination during meat handling, processing, and retailing due to poor personal hygiene and sanitary conditions (8).

Humans have learned from their experiences that eating the meat of diseased animals may lead to serious disease conditions. The importance of meat-borne diseases (MBDs) has been emphasized with the development of the meat industry (9). Meat-borne diseases can be of chemical or toxicological origin, zoonotic animal diseases, or environmental contaminations. Among these types, bacterial pathogens are the most important causative agents, whether as zoonotic diseases or environmental contaminations (10).

Several bacterial pathogens, including *E. coli*, *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Brucella* species, *Mycobacterium bovis, Bacillus anthracis* or toxin-producing species like *Staphylococcus aureus*, *Clostridium* species, and *Bacillus cereus*, cause meat-borne disease either by infecting animals or contaminating meat during meat processing or handling (11). Animals, the environment, human handlers, and contaminated water used during processing can be the sources of these pathogens. Therefore, preventing pathogens in food animals and having strict policies for proper hygiene are mandatory for minimizing MBDs (12).

Identification of the correct source of infection is usually hard to establish because of the slow progression of signs and symptoms of MBDs. The causative agent of the disease can be identified by testing a sample of recently consumed food.

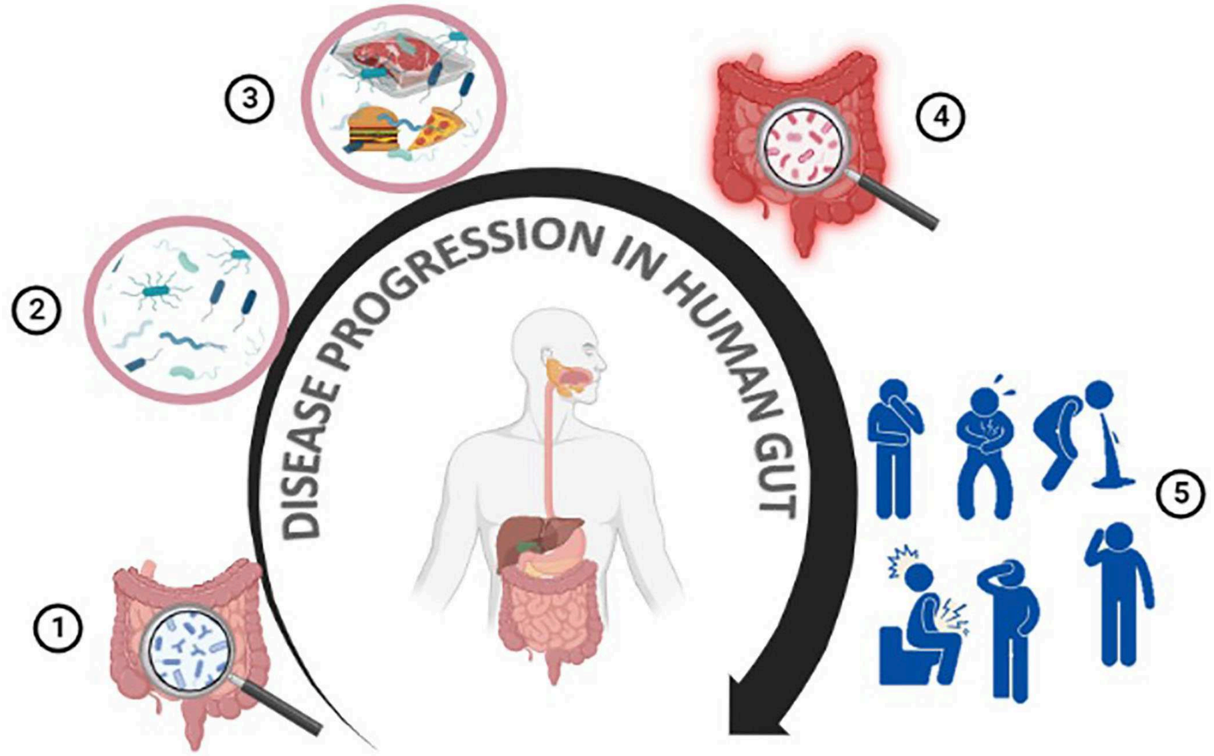


FIGURE 2
Meat-borne disease progression in humans. The human gut contains many beneficial/non-pathogenic microbes **(1,2)**. Meat or meat products can get contaminated at any point throughout the production, processing, or preparation steps **(3)**. Consuming contaminated food/meat or meat products can cause gastroenteritis, resulting in various health issues such as diarrhea, nausea, abdominal pain, and vomiting **(4,5)**.

However, it is hard to determine because the recently consumed food sample is not available for laboratory processing (13).

MBDs can be categorized into five types, i.e., infections, intoxications, allergies, metabolic food disorders, and idiosyncratic illnesses (14). Among these illnesses, infections and intoxications can affect almost every person. The remaining are comparatively less common. Consumption of contaminated meat can lead to various diseases that can be divided into GIT diseases and extra-GIT diseases. The bacterial pathogens causing GIT diseases include *E. coli*, *Salmonella*, *Campylobacter, Listeria*, and so on, while pathogens like *Brucella* and *Mycobacterium* can cause diseases other than GIT. Most cases of MBDs are due to gastrointestinal (GIT) problems, particularly small intestine issues that manifest as diarrhea.

Diarrhea

Diarrhea is a GIT disease that can be caused by a variety of pathogens and their toxins. Most meat-borne infectious pathogens cause exhausting diseases such as severe diarrhea. The term "diarrhea" comes from the Greek phrase "diarrhea," which means "to flow through." An increased bowel movement, fluid contents, and fecal volume distinguish it. Ultimately, unabsorbed solutes increase intestinal movement, and abnormal intestinal structure results in diarrhea. Toxins from bacteria and the virulence factors of bacteria that multiply can also cause diarrhea (15). Intestinal microbiota plays a key role in fighting off infections and keeping the body healthy.

In contrast, eating contaminated meat or meat products can cause severe inflammation of the gastrointestinal tract and a variety of related symptoms, such as nausea, vomiting, abdominal cramps, and diarrhea. A general mechanism of the food-borne GIT infection has been described in Figure 2. Bacterial infections can cause diarrhea through two distinct methods:

Toxigenic diarrhea

The physiological movement of the small intestine is disturbed by bacterial enterotoxins. By binding to epithelial cells, enterotoxins cause an increase in the secretion of electrolytes and a subsequent loss of water. This mechanism only results

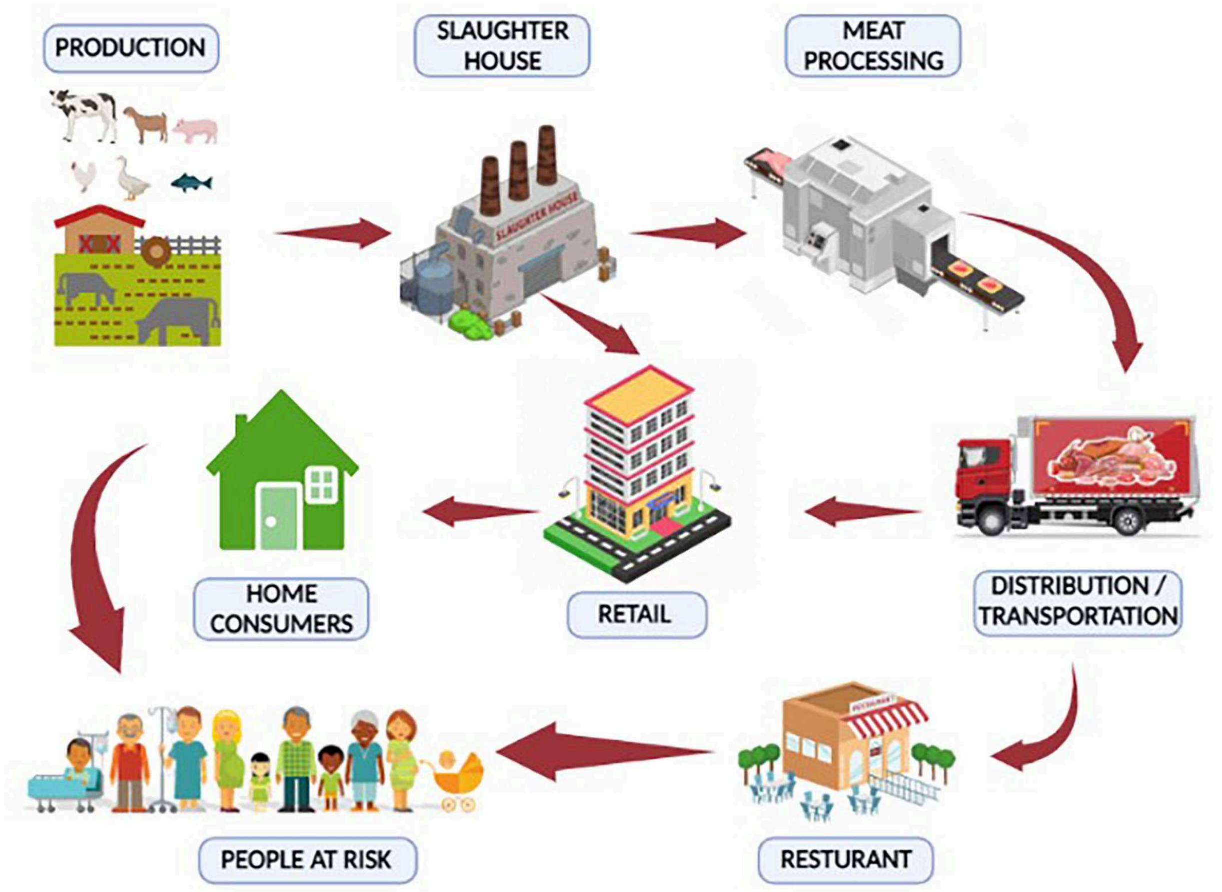


FIGURE 3
Meat supply chain (farm to fork). Meat or meat products can be contaminated with bacterial pathogens from "farm to fork". The meat supply chain includes a production system for animals, a slaughterhouse, a meat processing unit, transportation, a retail market, and a restaurant. Stringent control measures should be adopted to minimize contamination and, hence, meat-borne diseases.

in secretory diarrhea because bacteria do not penetrate beyond epithelial tissues (16).

Invasive diarrhea

The severe dehydration that results from invasive diarrhea, caused by pathogen infiltration of the epithelial tissues lining the small or large intestine, is a leading cause of death among children worldwide. Penetration into deeper tissues and epithelial lining ulceration can induce dysentery (the appearance of blood in feces) (17).

Meat-borne bacterial pathogens

Mesophilic and psychotropic bacteria typically contaminate red and white meat. The majority of MBDs are zoonotic and transmitted to humans by direct or indirect contact. Meat or meat products go through several processes in the meat supply chain before they are consumed by the final consumers (18). Meat and meat products can be purchased by consumers at grocery stores or consumed at restaurants. Many community members can get sick by consuming contaminated meat and meat products (19). An overview of the meat supply chain from farms to consumers has been described in Figure 3 (20). Considering the One Health strategy for effective disease prevention, this article reviewed the most prevalent meat-borne bacteria and the diseases they cause.

Escherichia coli

Escherichia coli (*E. coli*) is a gram-negative, rod-shaped, non-spore-forming, facultatively anaerobe that was first isolated from a fecal sample in 1885 by Theodor Escherich. It is part of the normal microbiota of human and animal intestines. *E. coli* strains are classified based on H-antigen (flagella), O-antigen (somatic), and K-antigen (capsule). Currently, there are 174

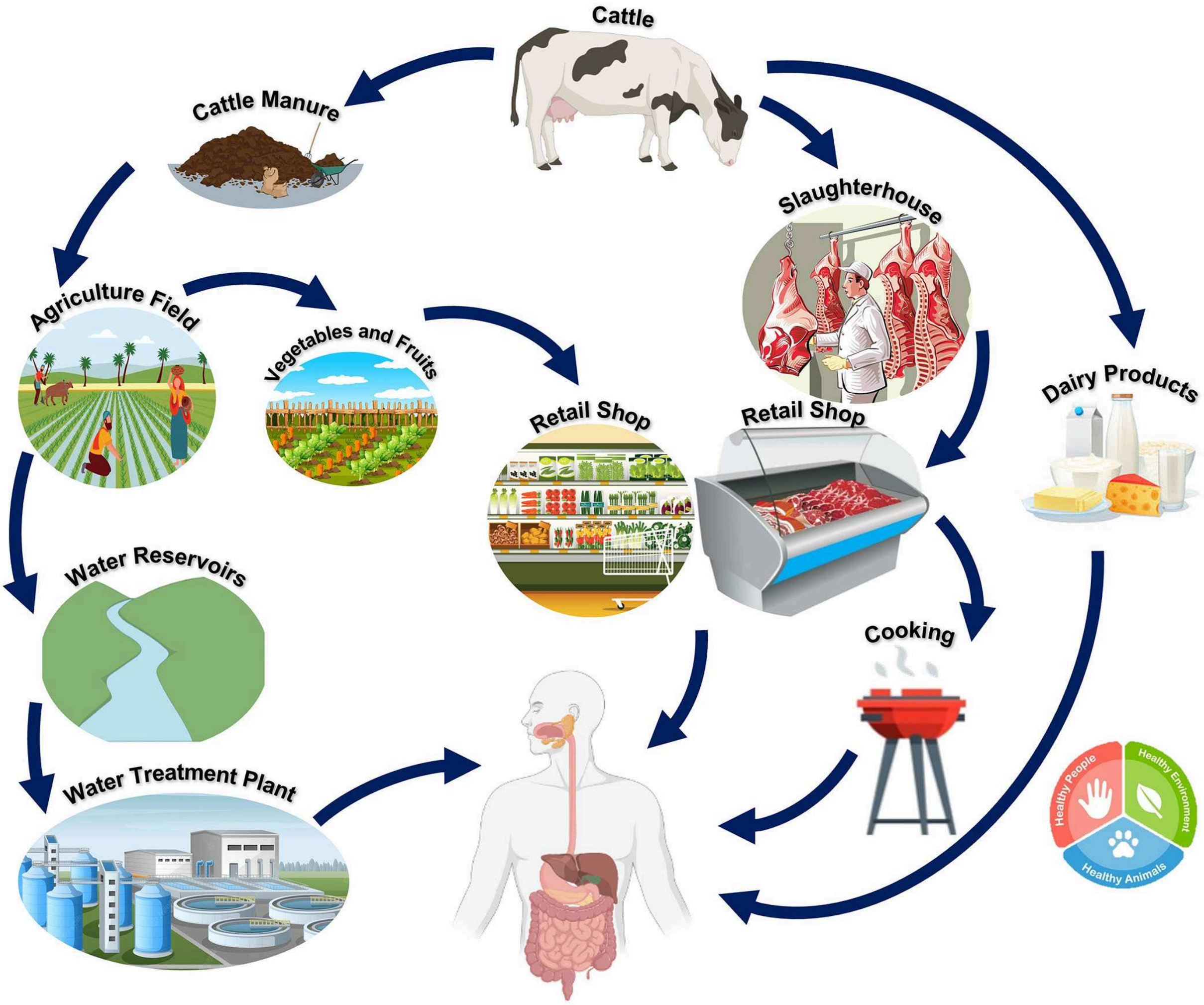


FIGURE 4
Reservoir and transmission of *E. coli* O157:H7. Cattle act as the main reservoir for O157:H7. The pathogen can be transmitted from cattle to humans directly (through the contamination of meat, meat, or dairy products from cattle) or indirectly [adapted from Vidovic and Korber (21)].

somatic antigens, 80 capsular antigens, and 53 flagellar antigens that have been reported (22).

Pathogenic *E. coli* serotypes are usually associated with diarrhea or intestinal illnesses, but some strains of *E. coli* can also cause non-intestinal diseases. The primary source of this pathogen is the animal population, which is transferred to humans through animal products (23). Since the human food chain remains the primary transmission route for *E. coli* O157:H7 infection in humans, it is essential to stress the role that an intermediate habitat (i.e., a natural environment, in particular, the human food chain) would play in the fate of the clinical strains (24). These clinical strains of *E. coli* O157:H7 in hospitals are thought to be significantly influenced by the intermediate habitat. This zoonotic pathogen has been demonstrated to survive in its intermediate habitat, including the natural environment and the food matrix, after being excreted from its primary habitat (cattle). In this period, the intermediate habitat promotes the evolution of *E. coli* O157:H7 strains that can endure the harsh conditions of the human food chain and the natural environment, increasing pathogen fitness (21). Figure 4 depicts the spread and continuous presence of *E. coli* in the environment and in humans and animals.

Many *E. coli* strains, once thought to be harmless, have acquired pathogenic genes and have evolved into potentially harmful pathogens. Such pathogenic strains, listed in Table 1, can infect humans and animals. Intestinal epithelial cell lining may be damaged and compromised by these pathogenic strains, which in turn can lead to disease by disrupting ion pumps, altering cytoskeletal assembly, triggering cell death, and exacerbating fluid loss (26).

TABLE 1 Pathotypes of pathogenic *E. coli*, diseases, and important virulence toxins.

***E. coli* pathogenic strains**	**Disease**	**Important toxins**
Intestinal pathogenic *E. coli*		
a. Enterohemorrhagic *E. coli* or Shiga toxin-producing *E. coli*	Diarrhea, hemorrhagic colitis, hemolytic uremic syndrome	Shiga-like toxins, enterohemolysin
b. Enterotoxigenic *E. coli*	Acute watery diarrhea	Heat stable toxin, Heat labile toxin, Shiga toxin
c. Enteroinvsive *E. coli*	Acute dysentery	Enteroaggregative heat stable, plasmid-encoded toxin
d. *Enteropathogenic E. coli*	Acute and/or persistent diarrhea	
e. *Enteroaggregative E. coli*	Persistent watery diarrhea	
f. Diffusely adherent *E. coli*	Watery diarrhea in children	Enterotoxin Secreted autotransporter toxin
g. Adherent invasive *E. coli*	Diarrhea, inflammatory bowel diseases	
Extraintestinal pathogenic *E. coli*		
a. Uropathogenic *E. coli*	Urinary tract diseases	
b. Sepsis-associated *E. coli*	Sepsis	
c. Neonatal meningitis *E. coli*	Meningitis in newborns	Hemolysin (Hly)
d. Avian pathogenic *E. coli*	Colibacillosis in fowls	Invasion (Ibe)

These data were adapted from Kaper et al. (25).

Food animals have been the source of several disease outbreaks in developed and developing countries. *E. coli* infections are a common source of reported GIT illnesses, and many of these cases are traced back to eating contaminated meat (27). Among *E. coli* strains, Enterohemorrhagic *Escherichia coli* (EHEC) can cause life-threatening diseases due to hemolytic uremic syndrome and hemorrhagic colitis. The EHEC strain, O157:H7, can be differentiated from other *E. coli* strains by its inability to ferment sorbitol. EHEC is believed to be present in a wide variety of meat, poultry, lamb, pork, and raw milk products (28). Strict monitoring in light of the One Health approach can help reduce the spread. Additionally, prevention strategies such as cooking, practicing good hygiene, and avoiding potentially contaminated food might reduce the prevalence of this bacterium in the general population (29).

Salmonella

Salmonellosis, caused by the bacterial pathogen *Salmonella*, is one of the most common causes of mortality globally. *Salmonella* is a gram-negative, rod-shaped, non-lactose fermenting, non-spore-forming, and facultative anaerobe belonging to the family *Enterobacteriaceae*. The species of this genus do best between 35°C and 37°C (30). Many serotypes of *Salmonella* have been identified, although they can be divided into two species: *Salmonella enterica* and *Salmonella bongori* (31). Serotypes belonging to the *S. enterica* subspecies enterica account for 1,586 of the 2,659 serotypes (32).

Salmonella serotypes can be classified into three groups according to their ability to infect different hosts: host-restricted, host-adapted, and generalist. Host-restricted serovars can infect only a single type of host, causing typhoid-like disease. These include *S.* Pullorum and *S.* Gallinarum (poultry pathogens) and *S.* Typhi and *S.* Paratyphi (human pathogens) (33). Host-adapted serovars normally infect a single host but can also infect other host ranges. These include *S.* Dublin (a cattle pathogen) and *S.* Choleraesuis (a pig pathogen) (34). Generalist serovars can live in the gastrointestinal tracts of a wide variety of animals, rarely causing systemic infections (35). Non-typhoidal *Salmonella* (NTS) serovars are particularly widespread among these, infecting a wide variety of animal and human hosts. These NTS include *S.* Enteritidis and *S.* Typhimurium (36). The World Health Organization (WHO) has declared that NTS is a major threat to world health, particularly in low-income nations (37).

After interacting with microfold cells, typhoidal *Salmonella* spreads to lymphoid tissue and causes a systemic infection. Eventually, they disseminate throughout the body *via* the lymphatic or circulatory systems. In contrast, NTS serotypes are localized to the intestinal tract and provoke a strong immune response (38). Overall, host-restricted serovars of *Salmonella* are more pathogenic than host-adapted and generalist serovars. The phenotype, genotype, and systemic impacts of these serotypes have been summarized in Figure 5.

Antimicrobial medications can inhibit infections by disrupting their key function. Nonetheless, antimicrobial resistance (AMR) can develop when bacteria find ways to evade these medications (39). The increasing prevalence of antibiotic-resistant bacteria due to horizontal gene transfer is a major public health threat (40). Multiple drug-resistance serovars have developed resistance to three or more different classes of antimicrobials (41). Five percent of NTS isolates from human infections have been observed to be resistant to multiple drugs (42). A major threat to global public health is posed by multidrug-resistant *Salmonella* serovars, which are now again on the rise (43). Using alternatives to antibiotics in the feed may help slow the spread of AMR in animals (44).

Especially in the case of host-adapted serovars, most *Salmonella* infections in farm animals are acquired from animals of the same species. There are notable behavioral differences between *S.* Dublin and *S.* Typhimurium in adult cattle. The cases of *S.* Dublin clinical infection that resolve in the animal

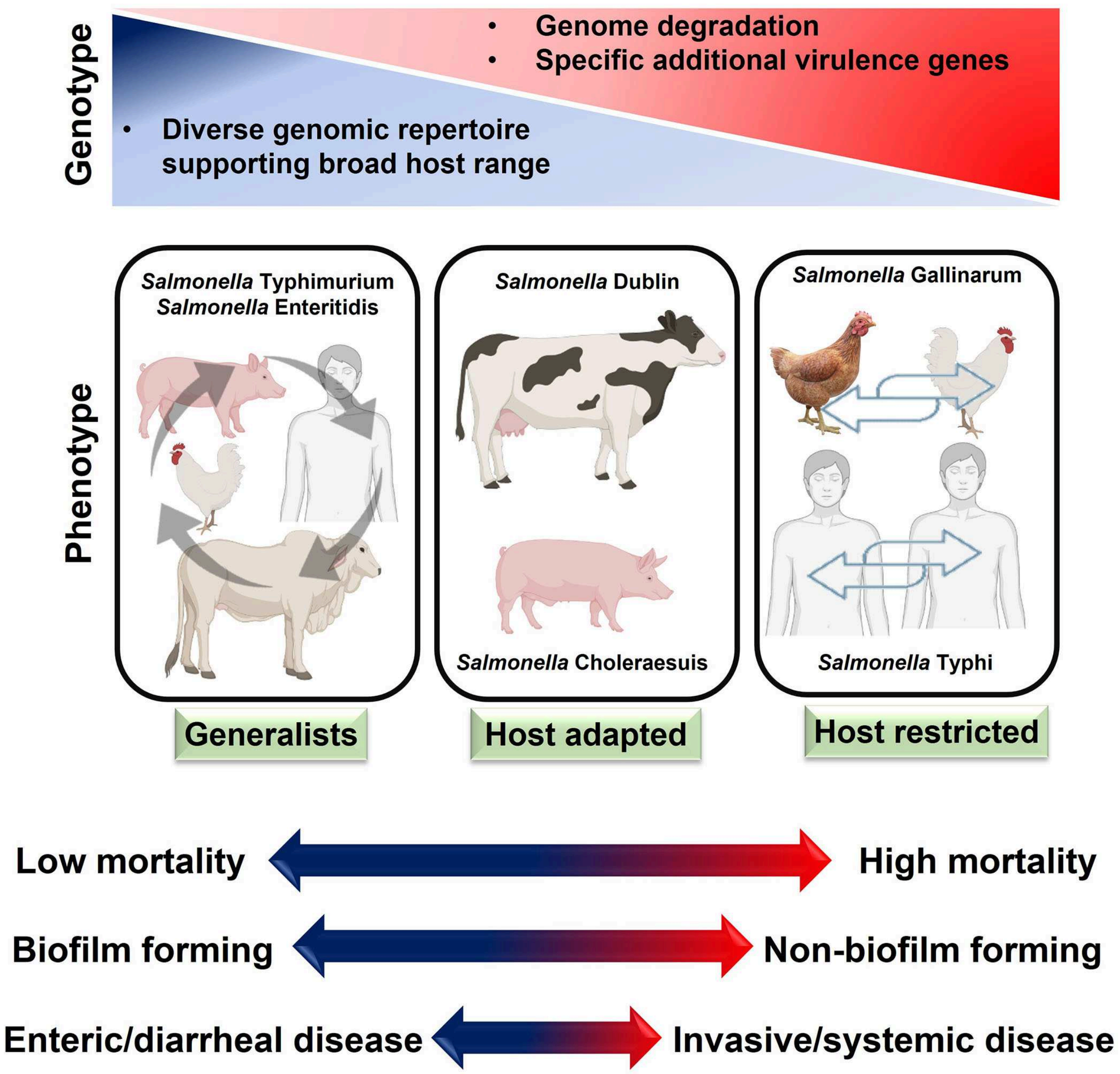


FIGURE 5
Host adaptation of salmonellae and disease characteristics. Generally, host-restricted salmonellae cause more severe systemic diseases than host-adapted and generalists *Salmonella*.

may become long-term carriers. It is possible that other herds are also infected but only show symptoms during times of high stress, especially during parturition (45). Experimental infection of calves by aerosol has also been reported, supporting the long-standing hypothesis that *Salmonella* may be transferred through the air (46). There have been numerous clinical cases in adult cattle because of grazing on recently contaminated pastures. Figure 6 summarizes how NTS can spread and persist at the point of contact between humans, animals, and their natural environments (47). Continuance of surveillance, early detection and management of sources, adequate hygienic measures, and execution of government rules and policies can help reduce the number of salmonellosis cases (48).

Campylobacter

Campylobacter, the bacteria responsible for campylobacteriosis, has a major impact on public health and the economy, especially in developing countries. It is gram-negative, curved, flagellated, microaerophile, catalase positive, and oxidase positive, and it can grow at an optimum temperature of 37–42°C. *C. jejuni* and *C. coli* are two of the

FIGURE 6
Reservoirs and transmission of non-typhoidal *Salmonella* (NTS). Farm animals serve as the main reservoirs for non-typhoidal *Salmonella*. The pathogen can survive in various animals or environments and eventually be transmitted to humans *via* consuming contaminated meat products or *via* direct contact with domestic animals.

most common species that cause disease in people. Researchers have linked these two species to both domestic and wild animals (49). *C. jejuni* is responsible for over 90% of all *Campylobacter* infections (50).

Poultry is a major natural reservoir of *C. jejuni*. Within poultry flocks, they spread through fecal-oral transmission (51). It is believed that only a low infectious dose (500–800) of *C. jejuni* is enough to induce GIT disease in humans (52). *C. jejuni* can contaminate water sources and thrive in domestic animals such as cattle and pork. Consumption of unpasteurized milk or undercooked meat can lead to GIT inflammation caused by *Campylobacter jejuni,* infecting the epithelial cells lining the intestine (53). The onset of the disease's symptoms might occur anywhere from 1 to 10 days after exposure. Patients with compromised immune systems are the only ones who are typically severely infected with *Campylobacter*-caused gastroenteritis (54). This pathogen can survive the hostile environment of the intestine due to various virulence factors, including motility, bile resistance factors, adhesion factors, and many different cytokines like cytolethal distending toxin (CTD) (55). Campylobacteriosis has a wide range of hosts and can be found in animal and environmental settings (see Figure 7 for an overview). Adaptation of proper hygienic practices during the handling of animals and animal products, such

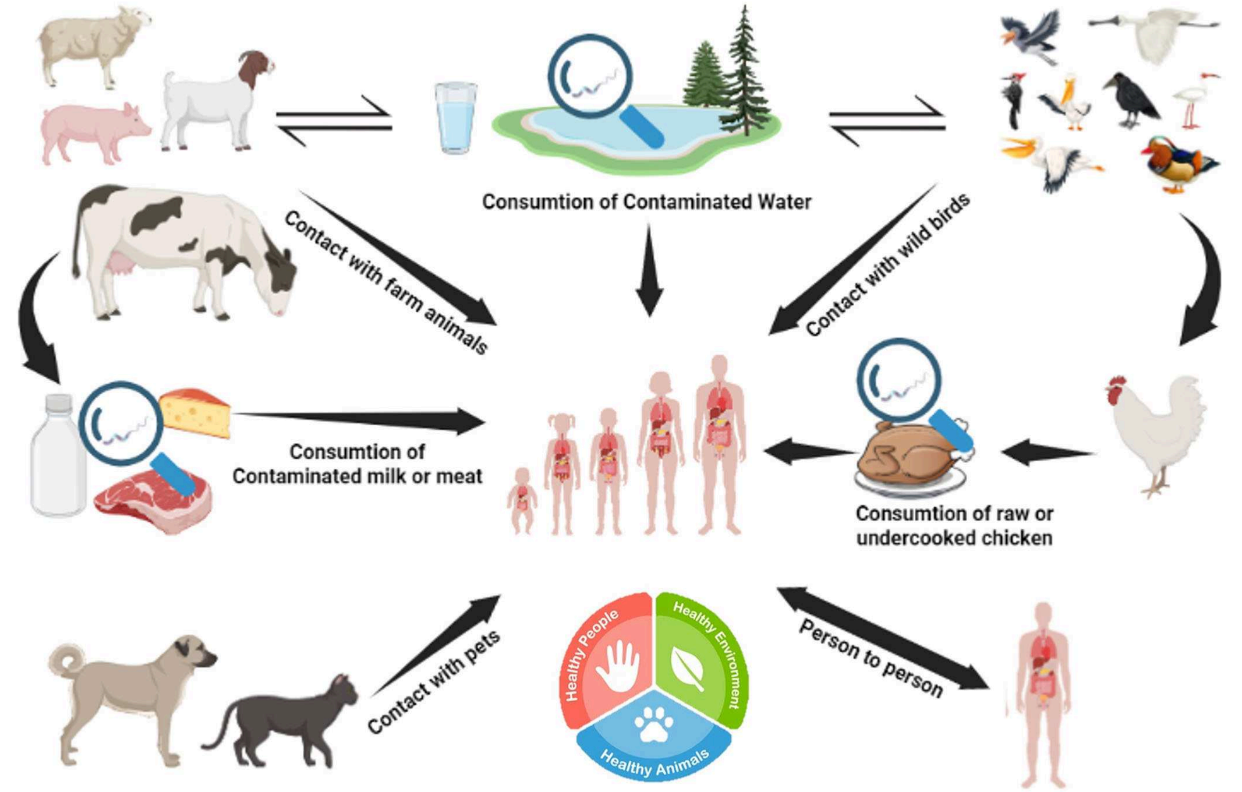


FIGURE 7
Reservoir and transmission of *Campylobacter*. *Campylobacter jejuni* can survive in various animals (poultry, cattle, and wild birds) and water reservoirs. Humans can acquire *C. jejuni* from consuming contaminated meat or meat products.

as fully cooked meat, can lower the risk of *Campylobacter* infections (56).

Shigella

Species of the genus *Shigella* are the pathogens that most often cause human dysentery. It is a gram-negative, rod-shaped, non-motile, non-lactose-fermenting, and facultative anaerobe pathogen. Table 2 shows how the pathogen has been classified into four categories based on the O antigen of the lipopolysaccharides. The genes for the lethal Shiga toxin are harbored in this pathogen (58). In immunocompromised patients, shigellosis can induce a severe form of the hemolytic uremic syndrome. *S. dysenteriae*, more than any other species, causes life-threatening shigellosis. It can be transmitted through the fecal-oral route due to poor sanitary practices. Only human beings are thought to harbor the pathogen (59). Using contaminated equipment, improper storage, and inadequate cooking can exacerbate the problem. The transmission of Shigellosis can be reduced by addressing the 5 "Fs," i.e., food, fingers, feces, flies, and termites (60).

Listeria monocytogenes

Even though there are several species in the genus *Listeria*, most of them are harmless because they lack the virulence factors that make their hosts susceptible to infection. Due to its capacity to cause disease (listeriosis) in humans and animals, *Listeria monocytogenes* has been classified as a public health issue. It is a gram-negative, rod-shaped, facultative anaerobe, glucose-fermenting, and able to grow at a wide range of temperatures (0–45°C) (61).

At first, it was thought to be responsible for causing abortions and encephalitis in rabbits and pigs. After 50 years, it was also established as a human food-borne disease. It has been established that it can induce gastroenteritis in humans, abortions in women, and meningitis in immunocompromised people. *Listeria monocytogenes* cause a significant fatality rate, between 20 and 30% (62).

Several large-scale listeriosis outbreaks have been linked to the consumption of ready-to-eat (RTE) meat, seafood, and dairy products. Listeriosis can be contracted by eating contaminated food or encountering infected animals (63). Occasionally, it can be passed on to newborns. Its pathogenicity is based on its

TABLE 2 *Shigella* serogroups and their characteristics.

Species	Serogroup	Number of serotypes	Geographic location	Typical characteristics
Shigella dysenteriae	A	15	Asia, Africa, Central America	Most severe dysentery with high mortality
Shigella flexneri	B	8	Common in developing states	Causes less severe dysentery than *S. dysenteiae*
Shigella boydii	C	19	Indian subcontinent mainly	Serologically different from *S. flexneri*
Shigella sonnei	D	1	Most common in developed stated	Causes mildest shigellosis

These data were adapted from Muthuirulandi Sethuvel et al. (57).

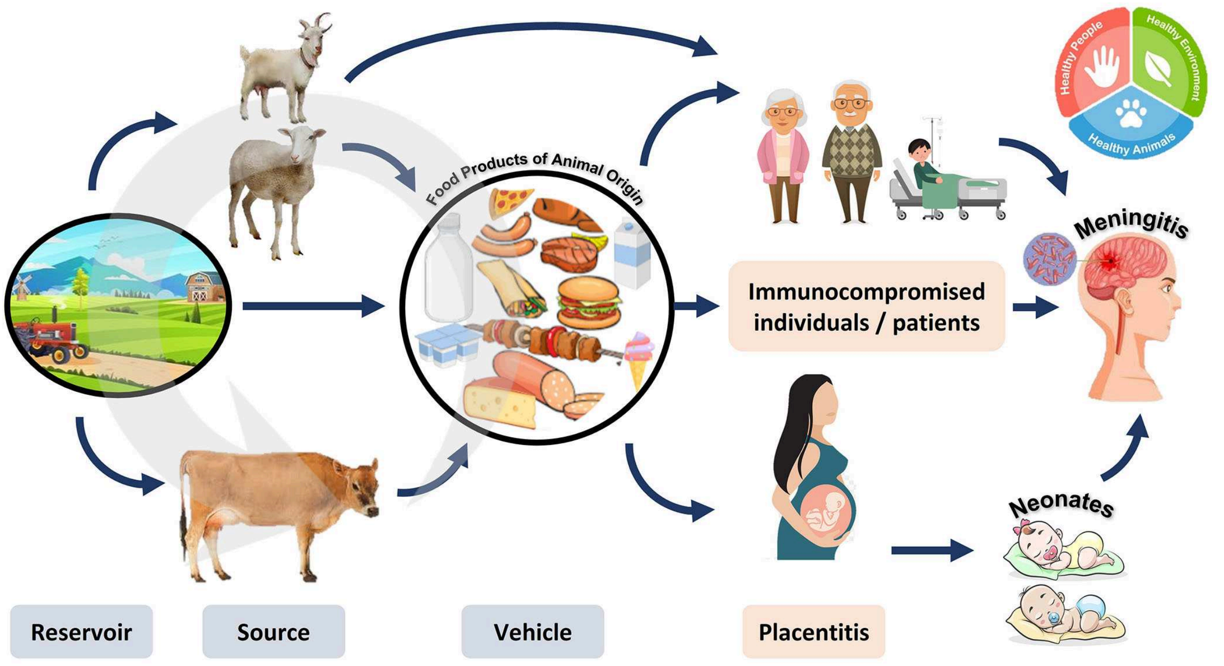


FIGURE 8
Reservoir, transmission, and diseases caused by *Listeria monocytogenes*. *Listeria monocytogenes* can circulate in animals and the environment. The pathogen can be transmitted to humans by consuming contaminated animal products. The resulting disease is more dangerous in pregnant women (causing inflammation of the placenta leading to abortion), neonates, and immunocompromised individuals (causing meningitis).

ability to proliferate within the cytoplasm upon phagocytosis. The survival of pathogens within phagocytes is ensured by the actions of listeriolysin O (LLO) and phospholipase (PlcA) (64). The pathogen can evade the humoral cell immune response by moving from cell to cell. Even in healthy people, listeriosis can cause a wide spectrum of symptoms, from mild diarrhea to deadly meningitis in those with compromised immune systems, as summarized in Figure 8 (65).

Yersinia enterocolitica

Yersinia enterocolitica, the causative agent of food-borne/meat-borne enteritis, along with *Yersinia pestis*, the causative agent of plague, and *Yersinia pseudotuberculosis,* belong to the genus *Yersinia*. Yersiniosis is caused by *Y. enterocolitica* and is often a self-limiting disease, but it can cause severe consequences post-infection in immunocompromised individuals (66).

After infecting small intestinal M cells, *Y. enterocolitica* travels through the body's lymphatic system. Antiphagocytic virulence factors are used to evade the host's immunological response. *Y. entercolitica* is capable of causing mild to moderate severity disease depending on host age, immune system condition, and environmental factors; however, it is rarely fatal (67).

Yersinia strains that are pathogenic to humans are primarily found in animal reservoirs, but they hardly ever cause illness in animals. Consumption of contaminated food, meat, and water can cause human disease. In addition, the waste products of food animals can potentially spread disease to humans when they contaminate fruit and vegetables (68). Figure 9 provides a concise summary of these animal food sources of the pathogen (69).

Brucella abortus

Brucella species are coccobacilli, which are Gram-negative, non-motile, aerobic, and non-spore-forming bacteria. These facultative intracellular bacteria exacerbate severe illnesses in animals and humans (70). Some of the most notable species in this genus and the animals that host them are *B. melitensis* (sheep and goats), *B. ovis* (sheep), *B. suis* (pigs), and *B. abortus* (cattle). Only *B. melitensis, B. suis*, and *B. abortus* can cause human brucellosis. *Brucella melitensis* is the most common species that causes brucellosis in humans, partly because of difficulties in immunizing free-ranging goats and sheep (71).

Brucellosis is common in many parts of the world, including Asia, South America, the Middle East, and Africa. Every year, more than half a million human cases are reported worldwide (72). Additionally, this is probably an underestimate because brucellosis cases are underreported and frequently misdiagnosed due to the lack of specific symptoms, the possible lack of awareness among physicians, and the limited diagnostic capabilities of laboratories (73). People having direct contact with animals, animal products, or people working in a laboratory with animals are at greater risk of exposure to brucellosis (74, 75).

Farmers, veterinarians, butchers, laboratory workers, milkers, and inseminators all risk contracting an infection at work due to their proximity to animals (76). Most field veterinary assistants, abattoir workers, and people working in many rural pastoral settings routinely handle aborted materials or attend to cases of retained placenta or dystocia without wearing protective gear. As a result, if the disease is present in domestic animals, it may also pose a significant threat to rural communities and animal health workers. Moreover, laboratory workers can accidentally acquire brucellosis while handling bacterial samples or cultures, as summarized in Figure 10 (77).

This pathogen only needs 10–100 cells to infect a human, but it can cause a debilitating, long-term illness (78). This pathogen can infect and replicate within a wide variety of host cells. Due to the fluctuating nature of the fever, this condition is also known as "undulant fever" when it occurs in humans. The disease is characterized by flu-like symptoms, such as a high temperature, a sore throat, a cough, a headache, a sore body, joint pain, sweating, fatigue, and anorexia (79). Adaptation of proper sanitary measures, safe handling of animals and animal products, and consumption of pasteurized milk can reduce the risk of brucellosis. It is imperative to re-establish a strict animal and human surveillance program by applying One Health principles (80).

Mycobacterium bovis

Since ancient times, people have known that tuberculosis can be transmitted between humans and animals. The bacterium *Mycobacterium tuberculosis* is responsible for most human tuberculosis cases. On the contrary, *M. bovis* is responsible for bovine tuberculosis. However, *M. bovis* can also infect humans because the disease can be transmitted from animals (81). The capacity of the *Mycobacterium* to replicate in a diverse range of hosts exacerbates the situation (82). To resist immunological clearance by the host, mycobacteria significantly modify the innate defense systems employed by the host immune system (83). Zoonotic tuberculosis can be transmitted from animals to humans through direct contact with infected animals, exposure to contaminated environments, and consumption of contaminated dairy and meat products (84). People who lack an efficient immune system have a greater chance of contracting the infection. As shown in Figure 11, the One Health approach should be used to reduce the number of infectious agents encountered in people, animals, and their environments (85).

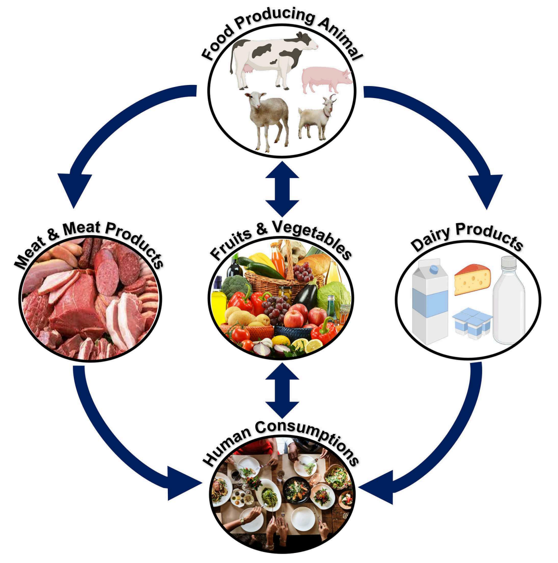


FIGURE 9
Transmission of *Y. enterocolitica* in humans. *Y. enterocolitica* can be transmitted to humans from animals through the consumption of contaminated food/meat or meat/dairy products.

Meat-borne intoxication

Toxins produced by pathogenic bacteria naturally found in or transmitted to meat or meat products can cause meat-borne intoxication (86). Some bacteria can survive high temperatures or enter food even after it has been cooked or pasteurized. During the food-borne multiplication of these pathogens, they can generate neurotoxins and enterotoxins. Bacterial pathogens like *Staphylococcus aureus, Bacillus cereus,* and *Clostridium perfringens* are examples of this group (87).

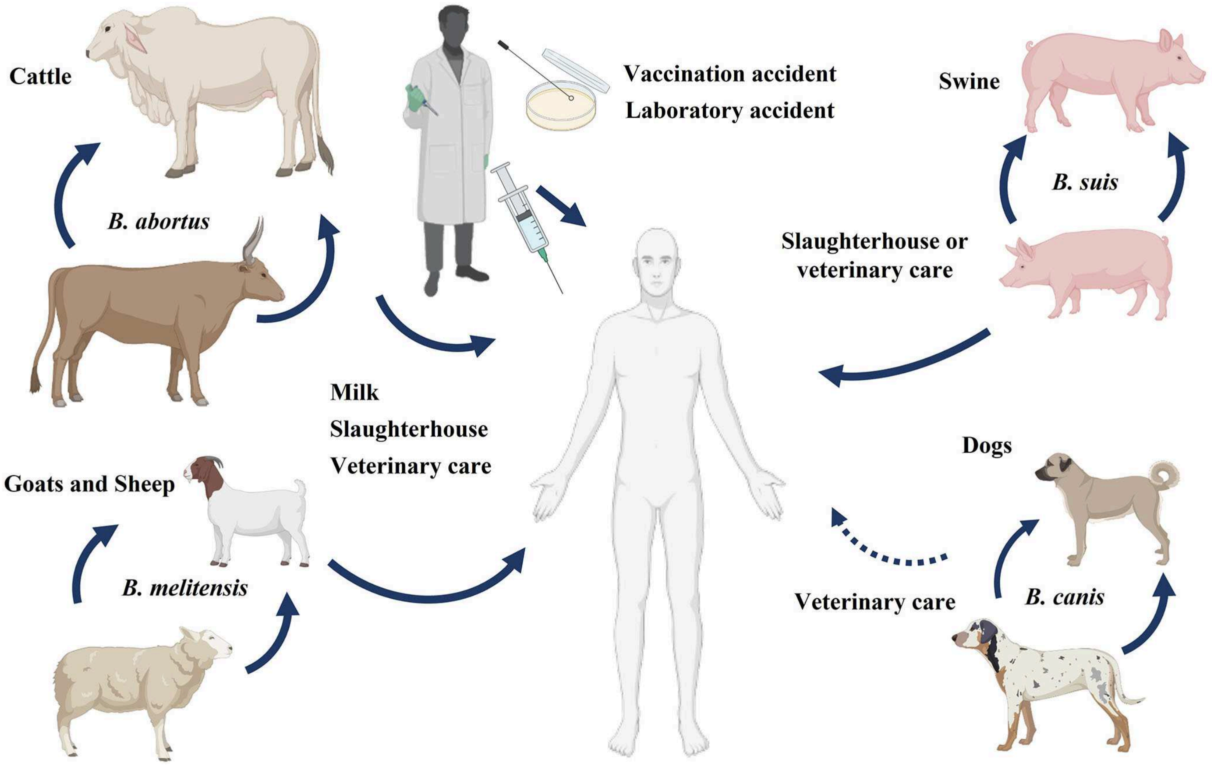


FIGURE 10
Reservoir and transmission of *Brucella*. Brucellosis in humans can be transmitted from different animals like cattle, sheep, goats, swine, and rarely from dogs. The sources of disease transmission include consumption of unpasteurized milk and milk products, direct association with animals or animal products, and accidental laboratory exposure.

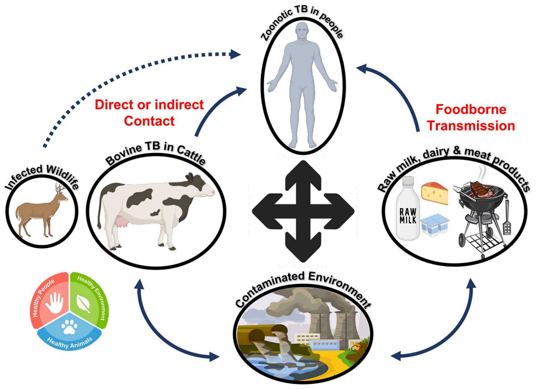


FIGURE 11
Reservoir and transmission of zoonotic tuberculosis. Bovine tuberculosis can be transmitted to humans directly or through contaminated dairy or meat products. The pathogen can survive in the environment and then enter the food chain, resulting in tuberculosis in humans after transmission.

S. aureus is the most common cause of food poisoning from contaminated meat.

Staphylococcus aureus

The food intoxication caused by a member of the *Staphylococcus* family is commonly termed staphylococcal food poisoning (SPF). There are more than 50 recognized species and subspecies of *Staphylococcus* (88). Staphylococcal species are classified as either "coagulase positive" or "coagulase negative" based on whether or not they produce the coagulase enzyme. The coagulase enzyme acts as a virulence factor by converting prothrombin into staphylothrombin and plasma fibrinogen into fibrin, helping bacteria evade the immune response (89).

S. aureus produces a diverse array of virulence factors and toxins. Among these factors, Staphylococcus enterotoxins (SEs) are responsible for food poisoning commonly produced by coagulase-positive *S. aureus*. Among the 23 different SEs, some cause pyrogenic disorders, enteritis, and food poisoning. These plasmid-mediated toxin genes can easily be transmitted horizontally to non-virulent strains, altering them into virulent strains (90). Staphylococcal toxins (A–E) are called classic enterotoxins except for SE–F due to their structural similarity with toxic shock syndrome toxins (91). The two most prevalent food poisonings are associated with SEA and SEB. The following Table 3 describes several different SEs along with their associated pathologies:

Consumption of SEs-contaminated meat and meat products causes food-borne staphylococcal intoxications (93). Depending on the sensitivity and immune condition of the affected person, as little as 0.1 μg of SEA toxin is enough to cause intoxication (94). Implementing proper hygienic practices during food processing and handling can reduce the risk of food-borne intoxication. To help reduce the risk of staphylococcal food poisoning, Figure 12 [adapted from (95)] summarizes its likely causes, transmission pathways, consequences, and preventative measures.

TABLE 3 Superantigens produced by *S. aureus,* along with associated pathology and genes.

Superantigens	Associated pathology	Associated gene
Enterotoxin A	Enteritis, food poisoning	*Sea*
Enterotoxin B	Enteritis, food poisoning	*Seb*
Enterotoxin C	Enteritis, food poisoning	*Sec*
Enterotoxin D	Enteritis, food poisoning	*Sed*
Enterotoxin E	Food poisoning	*See*
Enterotoxin G	Food poisoning	*Seg*
Enterotoxin H	Food poisoning	*She*
Enterotoxin I	Food poisoning	*Sei*
Enterotoxin F / TSST$_{-1}$	Toxic shock syndrome	*Tst*

These data were adapted from Fisher et al. (92).

One Health and food safety

Global increases in the production and consumption of animal products are inevitable, given proportional gains in wealth and technological advancements in livestock and poultry production. Concerns have been raised about the capacity of existing animal and public health infrastructures to support the Livestock Revolution's rapid expansion of animal agricultural production systems in developing countries (96).

The idea of "One Health" has been around for a while, but it has recently reemerged as a crucial framework for comprehending and responding to the health problems of the modern era. The term "One Health" refers to a multi-pronged approach to improving the wellbeing of humans, animals, and the environment. Our world is dynamic, intricate, and increasingly interconnected. Health in all three groups is now intricately intertwined owing to the unique dynamic formed by the confluence of humans, animals, and the environment (97).

The relationship between humans and animals, especially animal products, is evolving because of these shifting dynamics. Human interaction with animals is booming, spreading, and having greater and greater consequences. As a result, we now have an impressive global food system that is both an agricultural and business achievement and a tremendous challenge that endangers the health of humans, animals, and the environment (98). While improvements

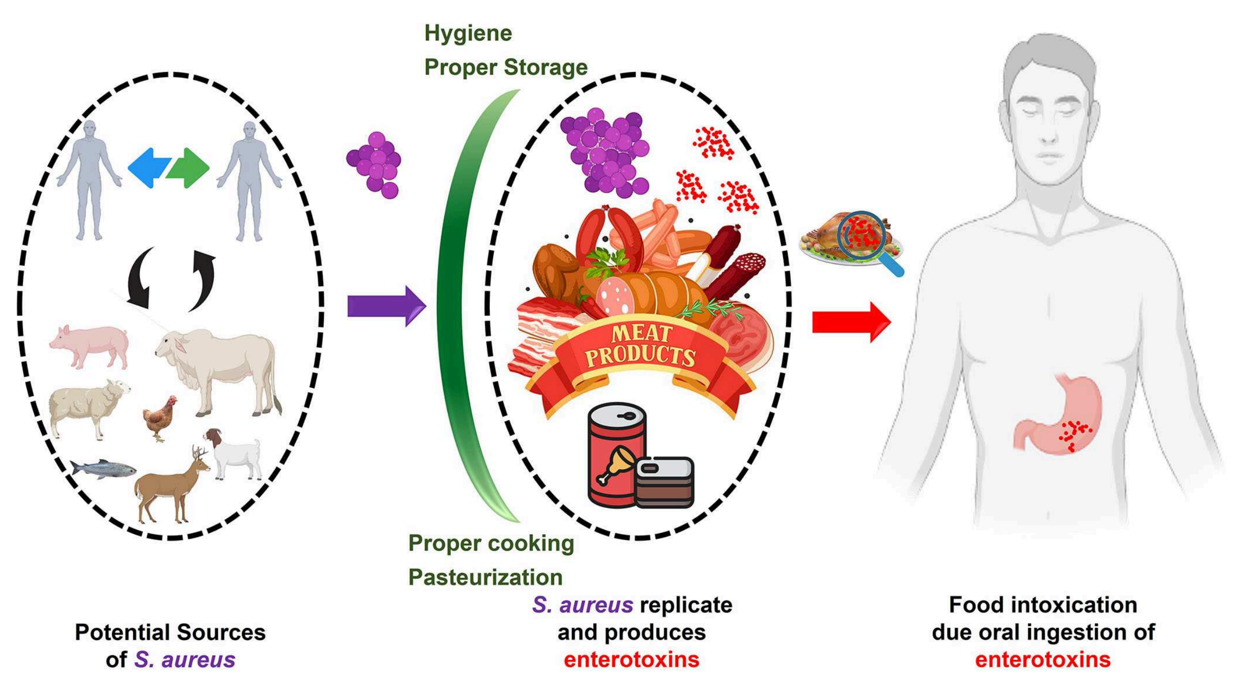


FIGURE 12
Staphylococcal food poisoning (SFP)/intoxication. *Staphylococcus aureus* **(Violet)** can enter food from various sources. Improper storage may lead to the replication of *S. aureus* and the production of enterotoxins **(Red)**. The consumption of enterotoxin-contaminated food (intoxication) may lead to gastroenteritis. Possible intoxication can be inhibited by proper cooking, pasteurization, and storage **(Green barriers)**.

in preventing and reducing food-borne diseases and other forms of food contamination have been made, they have been inconsistent, short-lived, and extremely challenging to sustain. New dimensions of cooperation, insight, and imagination will undoubtedly be required to guarantee a safe food supply (99). One Health is an attractive and contemporary approach because it takes a more proactive and integrated approach to finding and implementing solutions. It is imperative to create a new framework for preventing food-borne diseases rather than only reacting to them when they occur (100).

Embedded in this complex system is the potential role of food as a vehicle for disease transmission; food safety has grown in significance and become a vital public health commitment. This is a reminder that even though bacterial contamination of food is a major problem, other pathogens such as viruses, parasites, poisons, prions, chemicals, metals, and allergies can spread through consumption. Meat is gaining relevance as a vector for food-borne diseases; however, animal reservoirs are frequently the source of these infections. The concept of “One Health” provides the appropriate framework to examine this connection and, more significantly, gain a novel understanding that can help us modify our existing interventions and preventive measures (101).

For example, regarding the dangers of consuming certain types of food, we tend to concentrate on the potential effects on human health, even if the most efficient methods of preventing the spread of certain illnesses lie in the control measures taken. Microbes do not discriminate between species; they only want to survive and multiply. Our bias and artificial divide between animal and public health is a barrier to recognizing One Health. As a subset of this wider trend, the security of our food supply is becoming increasingly threatened by both deliberate and accidental contaminations and shifting microbial ecosystems (102).

The prevention and management of zoonotic diseases spread through meat can be enhanced by collaboration and cooperation under One Health concept. Because meat contamination can occur at various steps of the production and processing of meat, preventing meat-borne zoonotic diseases requires a comprehensive approach spanning from the point of production (at the farm) to the point of consumption (on the table). For example, cattle can be infected with *E. coli* from feed or the environment (at the farm), or beef can be contaminated with intestinal contents or the environment during slaughtering and processing (103).

Conclusion

Meat-borne infections are a serious threat to public health in both developed and developing countries due to the widespread consumption of contaminated meat and meat products. Proper hygienic standards and stringent production, processing, and handling precautions are required to limit the risk of meat-borne diseases due to the transmission of pathogens from animals to people. Animal disease control efforts that fail to address the underlying causes of the disease may increase the likelihood of AMR in bacterial pathogens. Because AMR is rising and poses a risk to public health, it is important to use antibiotics judiciously in animal production and treatment. Vaccination of food handlers and animals and the application of the Codex Alimentarius Commission should be followed to decrease the prevalence of food-borne pathogens and infections. One Health approach approved by WHO, FAO, and OIE can present solutions to reduce food-borne diseases concerning public health. In conclusion, political will is required to ensure that the agreed policies are implemented.

Author contributions

AA and SA co-wrote the review and approved the final draft. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Perception and determinants leading to antimicrobial (mis)use: A knowledge, attitude, and practices study in the rural communities of Odisha, India

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Background: Antimicrobial resistance (AMR) has emerged as one of the major public health issues globally. This cross-sectional study determined knowledge, attitudes and practices (KAP) regarding antimicrobial use and AMR among rural communities of Tigiria (Odisha), India.

Methods: A semi-structured questionnaire based on socio-demographic characteristics, antibiotics usage, awareness of antimicrobial resistance, healthcare utilization and quality of life were asked to the participants using an electronic device with Open Data Kit. Descriptive statistics, independent t-test and ANOVA were performed to analyze the variables.

Results: A total of 1,003 participants were surveyed in the study from 25 villages of Tigiria. About 44.47% (95% CI: 41.36–47.60) of study participants have heard about antimicrobial medicines and 14.75% (95% CI: 12.65–17.13) of participants were involved in buying antibiotics without prescription over the counter. Around 20.14% (95% CI: 17.72–22.78) of participants, stopped taking antibiotics before completing the full course. The physical domain was the most affected with low scores compared to other domains of quality of life (QOL). The QOL scores were found significant ($p < 0.05$) across age, gender, education and ethnicity.

Conclusion: The study documented a significant level of KAP regarding antimicrobial (mis)use in the study. It is essential that antimicrobial stewardship programs for various stakeholders and educational programmes must be initiated to increase awareness of people on antimicrobial resistance.

KEYWORDS

knowledge, attitude, practices, antibiotics, antimicrobial resistance

1. Introduction

The development of antibiotics has dramatically transformed medical care and resulted in a global decline in mortality and morbidity from infectious diseases (1). Due to the rise of antimicrobial resistance (AMR), the efficiency of currently used antimicrobial drugs is declining, making it more difficult and expensive to treat illnesses and harder to manage outbreaks. This has emerged as one of the major public health issues globally (2). Antimicrobial-resistant organisms (AMRO) have emerged globally as a result of microbial evolution, antimicrobial misuse and abuse in veterinary, human, and agricultural settings and poor infection control procedures, among other factors as key contributors to the global rise of AMR. This has resulted in difficult-to-treat infections, prolonged hospital stays, higher healthcare costs, and poor health outcomes (3, 4).

Developing nations in Asia and Africa are at greater risk of antimicrobial resistance (AMR), even though it's a matter of global concern. It is predicted that by 2050 over 10 million people would die from the disease worldwide including roughly 4 million people each from Asia and Africa, if no action is taken to stop the spread of AMR (5). The 68th World Health Assembly, which took place in May 2015, saw the adoption of a Global Action Plan (GAP) against AMR by its member nations (6). This strategy mandated that each nation create and carry out a National Action Plan for Antimicrobial Resistance (NAPAR) for combating AMR. The World Health Organization (WHO) is urging its member nations to adopt and put into practice the updated Model List of Essential Medicines, which divided antibiotics into the categories of Access, Watch, and Reserve (AWaRe) (7). The AWaRe categorization aims to increase access to antibiotics that can save lives and avoid the development of resistance from overuse of the selected priority medications.

In the Low and Middle-Income Countries (LMICs), a systematic study has revealed an increase of 77% in the rate of antibiotic consumption and an overall 114% rise in antibiotic consumption from 2000 to 2015 (8). During the year 2000–2010, a 76% rise in global antibiotic consumption was collectively contributed by BRICS countries, i.e., Brazil, Russia, India, China and South Africa (9). Unfortunately, India has one of the highest rates of antibiotic consumption with a massive 23% when compared to the other BRICS nations (9). According to a recent study in India, it is reported that the total defined daily doses (DDD) was 5,071 million in 2019 and the defined daily doses per 1,000 people per day (DID) was 10.4. Azithromycin was the most consumed antibiotic molecule (12.6%) followed by cefixime (10.2%) (10). As per the scoping report in 2017, more than 70% of the isolates of the Gram-negative bacteria, i.e., *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* as well as almost half of all *Pseudomonas aeruginosa* were resistant to fluoroquinolones and third generation cephalosporins. Gram-positive bacteria such as *Staphylococcus aureus* had a methicillin resistance rate of 42.6% while *Enterococcus faecium* had a vancomycin resistance rate of 10.5%. For ciprofloxacin, resistance rates among *Salmonella typhi* and *Shigella* species were 28% and 82%, respectively. Tetracycline resistance rates for *Vibrio cholerae* ranged from 17 to 75% in different parts of the country (11).

There is a lack of AMR research examining the complexity of upstream and downstream issues in a country like India, particularly in areas with limited resources. There is a need for generating evidence from well-designed studies from the community to execute policy development and implementation to prevent the irrational use of antibiotics and reduce the spread of AMR. The following study is a quantitative study that was conducted to map the knowledge relating to antibiotics, antibiotic resistance and antibiotic usage practices among the rural communities of Tigiria, Cuttack district of Odisha. Healthcare utilization and the quality of life from the study participants were also recorded during the survey.

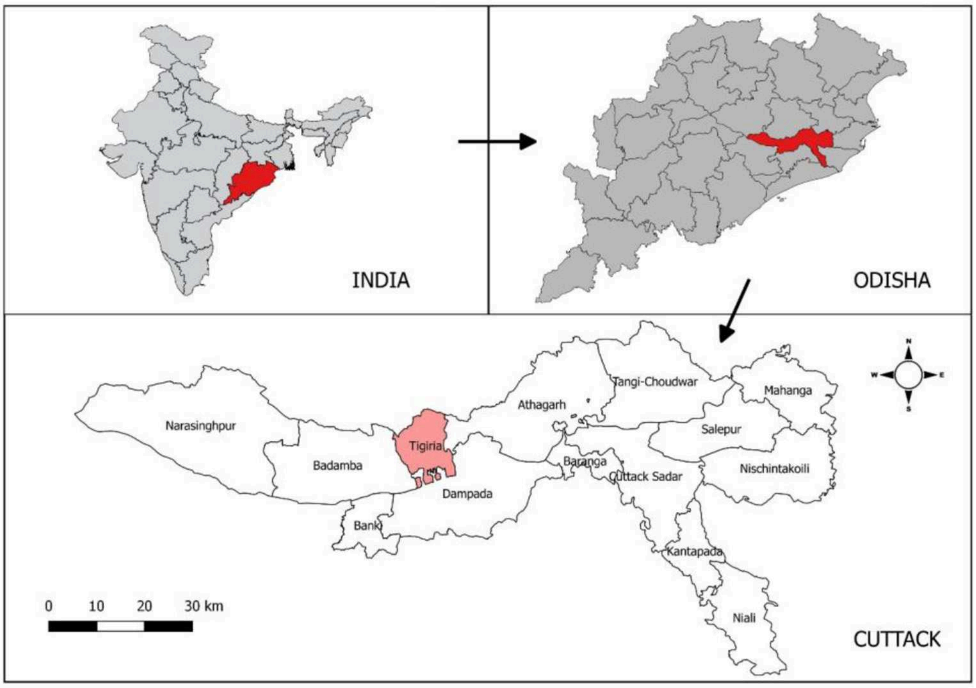

FIGURE 1
Study area site of the KAP in Odisha, India.

2. Methodology

2.1. Study design

It is a cross-sectional study conducted to assess the knowledge, attitude and practices prevalent among rural communities regarding antibiotic usage and antimicrobial resistance in the state of Odisha, India. This study was conducted from November 2021 to January 2021.

2.2. Study settings

The study was undertaken in the rural settings of Tigiria block ("Block" is a district subdivision), Cuttack district which is situated in the coastal area of Odisha with 50 villages under it. Tigiria block is located at 20°29′0″N latitude and 85°31′0″E longitude. Tigiria has a tropical savanna type of climate where the average summer temperature is 45°C and the average winter temperature is 10°C. Tigiria receives 964 mm of rainfall annually. Average relative humidity varies from 55 to 79% throughout the year. The total population of the Tigiria block is 75,000 according to 2011 census. The study area map is given in Figure 1.

2.3. Sample size

The sample size was calculated using the formula $(4pq/d^2)^{*}DE$, where p = awareness of antibiotics, $q = (1 - p)$, d = relative precision and DE = design effect. Assuming an awareness of antibiotics to be 50%, design effect of 1.3, relative precision of 4% and a non-response rate of 10%, the sample size was calculated to be 972 which was rounded off to 1,000.

2.4. Sampling method

Cluster sampling method was adopted for selecting study participants from the Tigiria block. There are 50 villages in Tigiria block out of which 25 villages were selected from the cluster sampling method. From each village, 40 households were selected for the survey. In each village, households were chosen through systematic random sampling method and only one adult individual was enrolled from each selected house. Simple random selection was used to choose a participant for the study if there were two or more adults living in a single household.

TABLE 1 Socio-demographic characteristics of the rural study participants.

Characteristics	Frequency (*n*)	Proportion (%)
Age		
18–45	457	45.6
45–60	331	33.0
60 and above	215	21.4
Gender		
Male	447	44.6
Female	556	55.4
Education		
No formal education	208	20.7
Less than primary school	357	35.6
High school	308	30.7
Secondary school	41	4.1
College/pre-university	73	7.3
Post graduate degree	16	1.6
Household size		
1–4	499	49.8
5–8	435	43.4
9 and above	69	6.9
Family type		
Joint	225	22.4
Single	60	6.0
Nuclear	710	70.8
Extended	8	0.8
Ethnicity		
General	264	26.3
Schedule caste	121	12.1
Schedule tribe	26	2.6
Other backward castes	592	59.0
Occupation		
Agriculture	246	24.5
Business	120	12.0
Daily labor	86	8.6
Housewife	383	38.2
Unemployed	59	5.9
Private job	54	5.4
Govt. job	21	2.1
Student	34	3.4

2.5. Data collection

An 86-item based questionnaire was developed to collect information from the study participants. The questionnaire consisted of semi-open questions based on socio-demographic characteristics, antibiotics usage, awareness of antimicrobial resistance, healthcare utilization and quality of life (WHO-QOL BREF scale) (12). The questionnaire was uploaded to electronic devices using an Open Data Kit. Questionnaire was developed in English and translated into the local language for a better understanding of the rural people. Hands-on training was provided to the field staff for collecting the data through electronic devices.

TABLE 2 KAP on antimicrobial usage and AMR.

Question	Response	Frequency (n)	Proportion (%)	95% CI
Heard about a medicine called antimicrobial medicine/antibiotics ($n = 1,003$)	Yes	446	44.47	41.36–47.60
	No	557	55.53	52.39–58.63
Knowledge of antimicrobial medicines ($n = 446$)	Azithromycin	96	21.52	17.85–25.69
	Amoxicillin	42	9.41	6.94–12.60
	Cefixime	4	0.90	0.28–2.44
	Cefpodoxime	1	0.22	0.01–1.44
	Doxycycline	1	0.22	0.01–1.44
	Ampicillin	1	0.22	0.01–1.44
	Other medicines	55	12.33	9.43–15.83
	Don't remember	283	63.45	58.77–67.89
Antibiotics were consumed for what diseases ($n = 446$)	Cold	125	28.03	23.95–32.48
	Sore throat	26	5.83	3.91–8.53
	Watery diarrhea	31	6.95	4.84–9.82
	TB	2	0.45	0.07–1.79
	Malaria	13	2.91	1.63–5.06
	Don't remember	249	55.83	51.07–60.47
Heard of the problem of antimicrobial resistance ($n = 1,003$)	Yes	1	0.10	0.005–0.06
	No	1,002	99.90	99.35–99.99
Asked for the antibiotics to the health professional on your own ($n = 1,003$)	Yes	18	1.79	1.09–2.87
	No	995	99.20	98.36–99.62
Took antibiotics without a prescription over the counter ($n = 1,003$)	Yes	148	14.75	12.65–17.13
	No	855	88.23	86.04–90.13
Stopped taking antibiotics before completing the full course ($n = 1,003$)	Yes	202	20.14	17.72–22.78
	No	801	79.86	77.21–82.27

2.6. Data analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 21 (IBM®, USA). Distribution of frequency, percentages of variables and 95% confidence intervals (CI) were calculated for categorical variables. Continuous variables were calculated as mean with standard deviation (SD). Independent t-test and one-way analysis of variance (ANOVA) were used to determine the association of socio-demographic variables with WHOQOL-BREF domains. All tests of significance were two-tailed with a $p < 0.05$ indicating a statistically significant difference. Study area map was made using QGIS v3.10 which is an open-source software freely available on the internet.

2.7. Ethical concerns

Ethical clearance was obtained from the Institutional Human Ethics Committee of ICMR-Regional Medical Research Centre (RMRC), Bhubaneswar (ICMR-RMRCB/IHEC-2019/034) and the State Ethical Committee of Odisha. Each participant was explained about the aims and objectives of the study and provided with the participant information sheet for reference. Written informed consent was taken from every participant before taking part in the study.

3. Results

A total of 1003 participants were surveyed in the study and most of the participants were from the 18 to 45 years age group. The proportion of female participants was more than the male participants. Around 20.7% ($n = 208$) of the study population received no formal education, whereas 30.7% of them have completed schooling (Table 1). About 50% ($n = 499$) of the houses consist of household members around 1–4. More than 70% ($n = 710$) of the houses had nuclear family structure, followed by joint family (22.4%). About 59% of the study population belonged to other backward castes, followed by the general population (26.3%). Occupation of these study participants were as follows in increasing order of their proportion, housewife (38.2%), agriculture (24.5%) and business (12%).

Almost 44.47% ($n = 446$) of study participants have heard about antimicrobial medicines (Table 2). About 21.52% ($n = 96$) of participants knew about azithromycin and 9.41% ($n = 42$) about amoxicillin among the participants who have heard about antimicrobial medicines. About 28.03% (95% CI: 23.95–32.48) of

TABLE 3 Healthcare utilization by the study participants.

Question	Response	Frequency (*n*)	Proportion (%)	95% CI
Sick for any time during the last 1 month ($n = 1{,}003$)	Yes	126	12.56	10.60–14.81
	No	877	87.44	85.18–89.39
Times you have been sick in the last 1 month ($n = 126$)	One	74	58.73	49.60–67.31
	Two	19	15.08	9.55–22.80
	Three or more	33	26.19	18.95–34.91
Your ailment each time ($n = 126$)	Fevers	64	50.79	41.78–59.75
	Weakness	70	55.56	46.45–64.31
	Body-aches	57	45.24	36.43–54.33
	Excessive fatigue and tiredness	15	11.90	7.04–19.17
	Headache	51	40.48	31.93–49.60
	Others	53	42.06	33.42–51.18
	Don't remember	7	5.56	2.45–11.52
Status of your ailment ($n = 126$)	Started more than 1 month ago and is continuing	32	25.40	18.25–34.06
	Started more than 1 month ago and has ended	32	25.40	18.25–34.06
	Started within 1 month and is continuing	23	18.25	12.15–26.34
	Started within 1 month and has ended	23	18.25	12.15–26.34
	Don't remember	16	12.70	7.65–20.09
Took any treatment for your illness ($n = 126$)	Yes	120	95.24	89.48–98.04
	No	6	4.76	2.04–10.30
Times, you have visited a health care provider for treatment ($n = 126$)	One	52	43.33	34.41–52.68
	Two	19	15.83	10.03–23.87
	Three or more	26	21.67	14.88–30.30
	Don't remember	23	19.17	12.77–27.57
Where did you go for treatment? ($n = 126$)	Government hospital/clinic	75	62.50	53.15–71.03
	Private hospital/clinic	26	21.67	14.88–30.30
	Pharmacist/dispensary	19	15.83	10.03–23.87
Accompanied you during each episode ($n = 126$)	None	30	25.00	17.74–33.88
	Spouse	38	31.67	23.64–40.87
	Son	24	20.00	13.47–28.49
	Daughter	1	0.83	0.04–5.23
	Son/daughter in-law	1	0.83	0.04–5.23
	Relatives	5	4.17	1.54–9.93
	Parents	5	4.17	1.54–9.93
	Don't remember	16	13.33	8.04–21.04
Who paid for your treatment? ($n = 126$)	Self	26	21.67	14.88–30.30
	Spouse	43	35.83	27.43–45.15
	Son	26	21.67	14.88–30.30
	Daughter	2	1.67	0.28–6.49
	Relatives	3	2.50	0.64–7.68

(Continued)

TABLE 3 (Continued)

Question	Response	Frequency (*n*)	Proportion (%)	95% CI
	Parents	8	6.67	3.13–13.12
	Don't remember	12	10.00	5.50–17.16
Reason for not taking any treatment ($n = 6$)	Facilities are available but lack of faith	1	16.67	0.87–63.51
	Financial reason	2	33.33	5.99–75.89
	Aliment not considered serious	1	16.67	0.87–63.51
	Others	2	33.33	5.99–75.89

participants took antibiotics for cold, 5.83% (95% CI: 3.91–8.53) for sore throat, 6.95% (95% CI: 4.84–9.82) for watery diarrhea, and 2.91% (95% CI: 1.63–5.06) for malaria. However, 14.75% (95% CI: 12.65–17.13) of participants were involved in buying antibiotics without prescription over-the-counter and 20.14% (95% CI: 17.72–22.78) of participants, stopped taking antibiotics before completing the full course. Similarly, 1.79% (95% CI: 1.09–2.87) of participants ask for antibiotics from health professionals on their own. Only 0.10% (95% CI: 0.005–0.06) of study participants knew about antimicrobial resistance.

Participants were also asked about their healthcare utilization, and it was seen that 12.56% ($n = 126$) of the participants were sick during the last month of the interview (Table 3). Out of those 126 participants, 74 (58.73%) got sick once, 19 (15.08%) got twice and 33 (26.19%) got sick thrice in the last month. Participants had different ailments such as fever 64 (50.79%), weakness 70 (55.56%), body aches 57 (45.24%) and headaches 51 (40.48%). About 120 (95.24%) of the participants went for pre-hospitalization and 75 (62.50%) went to a government hospital/clinic for treatment.

The mean score across the four domains and the overall domain of WHOQOL-BREF based on sociodemographic factors was shown in Table 4. QOL of younger age participants ($\leq$45 years) were observed with better scores significant ($p < 0.05$) in all their domains. Male participants had better QOL in physical, social and overall domains which were statistically significant ($p < 0.05$). With regard to education, illiterate participants had poor QOL in all domains when compared to educated participants ($p < 0.05$). Similarly, nuclear/single families displayed better QOL than joint families only in the social domain ($p < 0.05$). The QOL scores were found significant ($p < 0.05$) across ethnicity.

4. Discussion

This study represents one of the attempts to assess the knowledge, attitude and practices of antimicrobial use and antimicrobial resistance in a rural setting in the eastern region of India. It is expected that the findings from the present study will provide baseline information related to understanding the use or misuse of antibiotics in rural Indian communities. However, there is insufficient knowledge about antimicrobial resistance in the rural community. The level of knowledge observed in our study about antimicrobials and their use is similar to the findings from Jordan and Nepal (13, 14). The lack of knowledge regarding antibiotic use and antimicrobial resistance is in accordance with the previous findings (13, 15). An increase in participants' education is an important criterion that depicts their knowledge gain about antibiotic use and antimicrobial resistance which was reported in the findings from earlier studies (13, 15–17). More than 50% of the participants were unaware of AMR similar to the finding reported in Nepal (14). The level of knowledge on AMR seen in our study, however, is lower than that reported in Norway (18). Very few studies have been done among the general population to determine their knowledge of AMR as seen in the literature. Studies have primarily focused on individuals or specific groups such as medical professionals, veterinary or students (17, 19, 20).

In the study, it was observed that participants have heard about antibiotics but as many of them have not received complete education, they were not able to tell the antibiotic medicines, they had used. Nearly one-seventh of the participants preferred taking antimicrobial medicines without a prescription over the counter or without consultation with healthcare workers. This was an unhealthy method of taking antibiotics in the rural population. Participants directly asking the health professionals to prescribe antibiotics was minimal. Very few people stopped taking the antibiotics before completing the full course which might be due to their compliant nature with the healthcare workers or for the fear of not getting completely cured. However, the knowledge of antimicrobial resistance in the rural community is almost negligible when compared with the other studies (13–16).

In the community, AMR is primarily caused by self-medication or inappropriate use of antimicrobials (21). However, in these settings, evidence-based antimicrobial stewardship programmes are very limited. Education must be a key element of antimicrobial stewardship programmes in order for them to be successful. Healthcare workers must be involved in antimicrobial stewardship programmes so that antibiotics can be preserved, AMR and substantial morbidity and mortality can be reduced. The distinctive legal and cultural traits of the study populations should be taken into account while designing intervention plans. The intervention can only be successful when both the healthcare workers and community work as a whole to reduce AMR.

Inadequate awareness of antibiotic use and AMR should be seen as worrying issues and possible signs of AMR that must be addressed right away. The data from this study might be used by policy- and decision-makers as one of the inputs to monitor drug usage policy and recommendations and reduce the risk of AMR. Our findings advocate for customized treatments, such as community-focused education campaigns about optimal antibiotic usage and AMR prevention strategies. For example, persons with less formal education, who have shown less understanding and poor practices

TABLE 4 WHO-QOL BREF scores based on socio-demographic variables.

	Physical	Psychological	Social	Environmental	Overall
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Total	46.35 ± 7.36	65.37 ± 10.17	71.58 ± 8.61	64.12 ± 9.31	61.86 ± 6.20
Age					
≤45 years	47.55 ± 6.66	67.69 ± 9.22	72.98 ± 6.35	65.25 ± 8.84	63.37 ± 5.18
>45 years	45.02 ± 7.86	62.81 ± 10.56	70.03 ± 10.35	62.86 ± 9.66	60.18 ± 6.78
p	<0.001	<0.001	<0.001	<0.001	<0.001
Gender					
Male	47.14 ± 7.15	65.35 ± 9.86	72.36 ± 7.64	64.63 ± 8.56	62.37 ± 5.45
Female	45.71 ± 7.46	65.39 ± 10.42	70.94 ± 9.27	63.70 ± 9.85	61.43 ± 6.72
p	0.002	0.94	0.009	0.11	0.017
Education					
No formal education	43.13 ± 8.01	61.73 ± 11.01	69.13 ± 10.70	60.89 ± 10.33	58.72 ± 7.37
Received education	47.19 ± 6.94	66.33 ± 9.72	72.22 ± 7.86	64.96 ± 8.84	62.67 ± 5.58
p	<0.001	<0.001	<0.001	<0.001	<0.001
Occupation					
Agriculture	46.07 ± 6.05	64.95 ± 9.51	72.51 ± 7.64	63.72 ± 8.82	61.81 ± 5.39
Housewife	46.45 ± 7.34	66.28 ± 10.73	71.40 ± 8.62	64.28 ± 9.60	62.10 ± 6.32
Others	46.43 ± 8.13	64.72 ± 9.95	71.14 ± 9.15	64.21 ± 9.33	61.63 ± 6.57
p	0.78	0.08	0.13	0.74	0.57
Household size					
1–4	46.15 ± 7.53	64.86 ± 9.88	71.39 ± 8.58	63.58 ± 9.51	61.49 ± 6.15
5 and above	46.55 ± 7.19	65.89 ± 10.43	71.76 ± 8.64	64.65 ± 9.08	62.21 ± 6.24
p	0.38	0.11	0.49	0.068	0.066
Family type					
Nuclear/single	46.52 ± 7.35	65.42 ± 9.58	72.10 ± 8.03	64.31 ± 8.68	62.09 ± 5.60
Joint	45.93 ± 7.37	65.27 ± 11.48	70.30 ± 9.76	63.63 ± 10.67	61.28 ± 7.42
p	0.25	0.83	0.003	0.28	0.06
Ethnicity					
General	46.80 ± 6.83	66.87 ± 9.79	72.18 ± 7.58	65.57 ± 8.63	62.85 ± 5.88
Other backward classes	46.05 ± 7.39	65.46 ± 10.34	71.34 ± 9.00	64.20 ± 9.45	61.76 ± 6.36
Schedule caste	46.30 ± 7.84	62.29 ± 9.79	71.26 ± 8.96	60.82 ± 9.54	60.17 ± 5.88
Schedule tribe	48.80 ± 9.01	62.53 ± 8.37	72.34 ± 7.74	62.61 ± 7.82	61.57 ± 5.49
p	0.18	<0.001	0.55	<0.001	0.001

about the use of antibiotics and steps to combat their resistance, should be the focus of education programmes.

The healthcare system was efficiently utilized by the study population across all age groups. It shows that the patients had better access to the healthcare system showing higher utilization in the rural area which might be due to multiple factors such as better transportation, etc. The physical domain of life analysis, which addressed working ability, daily activities, bodily discomfort, sleep and rest, mobility and energy received the lowest score as per the WHO-QOL BREF scale. The QOL scores in other domains were higher than the physical domain and were found to be significant across age, gender, education and ethnicity.

There are very few studies have been conducted on antimicrobial use and AMR in rural settings, especially in India which is one of the major advantages of our study. The sample size is also big and generalizable inferences can also be drawn from a similar setting. However, the study has some limitations such as the data might have been affected by recall bias or fear among rural study participants of not sharing undesirable practices. To draw causal inferences from this study is not possible as the study is cross-sectional in nature. Besides these limitations, the data from the study can provide baseline information that can help to carry out future research and track the efficiency of intervention studies.

5. Conclusion

This study provides information on knowledge, attitude and practices on antimicrobial usage and AMR from rural settings of Tigiria, Odisha. The study documented a low level of knowledge and a high level of practice on antimicrobial usage and AMR. The findings are crucial for directing policy development, programme planning, in execution of antimicrobial use and AMR-related initiatives focusing the community-based awareness, education, and sensitization.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Ethical Committee, RMRC, Bhubaneswar. The participants provided their written informed consent to participate in this study.

Author contributions

SPat, DB, KW, and TS were involved in the concept, planning, and formulation of the study. AN, SKar, SS, DP, and HC were involved in data collection. Data analysis and the initial draft were prepared by MP. SPal, SKan, and JK reviewed the manuscript. All the authors have read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Carbon sequestration potential of different forest types in Pakistan and its role in regulating services for public health

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A high amount of CO_2 causes numerous health effects, including headaches, restlessness, difficulty in breathing, increased heart rate, high blood pressure, asphyxia, and dizziness. This issue of increasing atmospheric CO_2 can only be solved *via* above-ground and below-ground carbon sequestration (CS). This study was designed to determine the relationship between CS with the crown area (CA), diameter at breast height (DBH), height (H), species richness (SR), and elevation in different forest types of Pakistan with the following specific objectives: (1) to quantify the direct and indirect relationship of carbon sequestration with CA, DBH, H, and SR in various natural forest types and (2) to evaluate the effect of elevation on the trees functional traits and resultant CS. We used the linear structural equation model (SEM) for each conceptual model. Our results confirmed that the highest CS potential was recorded for dry temperate conifer forests (DTCF) i.e., 52.67%, followed by moist temperate mix forests (MTMF) and sub-tropical broad-leaved forests (STBLF). The SEM further described the carbon sequestration variation, i.e., 57, 32, 19, and 16% under the influence of CA ($\beta = 0.90$ and P-value < 0.001), H ($\beta = 0.13$ and p-value $= 0.05$), DBH ($\beta = 0.07$ and p-value $= 0.005$), and SR ($\beta = -0.55$ and p-value $= 0.001$), respectively. The individual direct effect of SR on carbon sequestration has been negative and significant. At the same time, the separate effect of CA, DBH, and H had a positive and significant effect on carbon sequestration. The remaining 20% of CS variations are indirectly influenced by elevation. This means that elevation affects carbon sequestration indirectly through CA, DBH, H, and SR, i.e., $\beta = 0.133$ and P-value < 0.166, followed by $\beta = 0.531$ and P-value < 0.001, $\beta = 0.007$ and P-value < 0.399, and $\beta = -0.32$ and P-value < 0.001, respectively. It is concluded that abiotic factors mainly determined carbon sequestration in forest ecosystems along with the elevation gradients in Pakistan. Quantifying the role of various forest types in carbon dioxide (CO_2) reduction leads to improved air quality, which positively impacts human health. This is an imperative and novel study that links the dynamics of the biosphere and atmosphere.

KEYWORDS

trees functional traits, carbon sequestration, elevation, structural equation model, regional scale, air quality

1. Introduction

The forest ecosystem provides clean air through carbon sequestration for better public health. The sequestered carbon is not emitted into the atmosphere, reducing the chances of various human diseases (1). Forest ecosystems determine global climate change by removing or adding greenhouse gases such as CO_2 from the atmosphere. Forests, alongside the grassland and peat swamps, collectively store more carbon than other terrestrial ecosystems (2). Forest trees store CO_2 from the atmosphere in the form of wood, soil organic carbon, and other biomasses which contribute to a reduction in global warming and climate change (3). Disturbances in these ecosystems due to the overexploitation of resources cause the release of a significant amount of CO_2 back into the atmosphere (4).

Carbon sequestration is essential to mitigate global climate change (5). Approximately 53% of carbon is stored in temperate and boreal regions, while the remaining 37 % is stored in tropical areas (6, 7). Half of the terrestrial carbon sink is located in forest ecosystems (8). Forest ecosystems take a large amount of CO_2 from the atmosphere *via* photosynthesis. They also contribute a large amount of fixed carbon in the form of organic matter in the lithosphere (9). However, a small portion of integrated carbon has been stored in the belowground biomass, litter, and soil (10). According to United Nations Food and Agriculture Organization (FAO) statistics, forests contain 234 Pg of carbon in the above-ground forest, 62 Pg below ground, 41 Pg in the form of deadwood, 23 Pg in litter form, and 398 Pg in the forest soils (11). Forest ecosystems occupy the most significant part of the free-ice land surface in the entire terrestrial world. Trees are the main component of the forest ecosystem and contain the entire quantity of forest living biomass. Forests' total biomass is ∼677 Petagram (PgC), and trees contribute about 80% of the world's total biomass (11).

Several biotic and abiotic factors influence carbon sequestration in forest ecosystems (12–14). Trees continuously sequester CO_2 from the atmosphere and store it in their different parts, i.e., stem, root leaves, and branches (15). The carbon sequestration rate depends on plant growth, individual characteristics of the tree species, the wood density, and its growing conditions. Trees store most of the atmospheric carbon because of their larger size, volume, and long-lived storage capacities, i.e., tree trunks, leaves, roots, and the soils in which they exist (16). Carbon sequestration is mainly driven through huge tree biomasses (17).

The scientific community is committed to reducing atmospheric CO_2 emissions and storing them in any other form to improve the quality of the atmosphere (18). Various biological and geological mechanisms bring free carbon down from the atmosphere. However, due to the enormous rise in human population, there is a continuous degradation of the land and deforestation, which results in the irreversible loss of forest functions. Plants are the primary producers of the world's biodiversity. They capture atmospheric CO_2 and convert it into glucose, the first organic molecule of life (19). Degraded forests lead to low carbon storage and poor biodiversity, which in turn cause global warming and climatic changes (20). Anthropogenic activities drastically add massive amounts of greenhouse gases, mainly $CO_{2,}$ into the atmosphere (16). The rapid increase in the concentration of CO_2, methane, and methane dioxide is the major cause of global warming (21).

Characteristically, above-ground biomass decreases along the elevation, but precipitation influences the biomass variations alongside the elevation gradient (22). Carbon sequestration is mainly determined by the biotic determinant of trees, such as variations in diameter, height diversity, stand density, and stand basal area (20, 23). The individual tree height is not associated with diameter, but the ratio is correlated to the species' genetic nature, intraspecific competition, and abiotic drivers like precipitation, temperature, and soil types (24–26). However, tree heights decline with increasing elevation, generally (27). Initially, researchers use DBH data to estimate carbon sequestration, but the precision of carbon estimation has improved using tree height data (28, 29). Proper forest administration and land improvement approaches that concurrently enhance the biodiversity and carbon stock to mitigate climate change and global warming across regional and global scales are required to bring positive changes at least at the micro-environment levels (30). However, the relationship between forest stand structure, i.e., height, diameter at breast height, crown area, and species richness along regional level elevation gradients in different forest types, have rarely been evaluated. Therefore, the current study was designed to quantify the carbon sequestration potential of various forest types in Pakistan, focusing on its biotic complexities.

1.1. Theoretical framework

The net primary productivity theory explains how forest productivity is affected by forest structure, i.e., First, the net immediate productivity drops during stand expansion due to the limited supply of water and nutrients to leaves within a forest stand. Second, how productivity changes systematically with climate change interaction and withstands developmental processes. These arguments provide predictions about variability in wood production, biomass loss rate, and carbon sequestration. The literature shows that the forest ecosystem is driven by biotic and abiotic factors such as forest age, forest origin, forest soil conditions, and geography of the region. Forest structure and growth are controlled by vegetation cover. Through aerial expansion, trees increase their potential height, producing more biomass (31, 32). The effects of abiotic and biotic factors on carbon sequestration at large regional-scale forest ecosystems have rarely been studied. Thus, the driving biotic and abiotic factors of carbon sequestration in multiple forest types are vague and need to be explored.

Stand structure is associated with differences in species composition, and there is a positive relationship between functional and taxonomic species diversity with the above-ground biomass (23, 33). The relationship between carbon sequestration with tree crown area, diameter at breast height, height, elevation, and species richness is usually positive. Still, the association and simultaneous direct and indirect impacts in different forest ecosystems remain questionable. Therefore, current studies were conducted in diverse forest ecosystems to evaluate the role of various biotic and abiotic factors on carbon sequestration at a regional level.

The most critical goal of the current work was to determine the direct and indirect role of CA, H, DBH, and SR on carbon sequestration in the diverse natural forests of Pakistan. Thereby, we also examine the effect of elevation on Carbon sequestration. We hypothesize that carbon sequestration is driven by CA, DBH, H, and

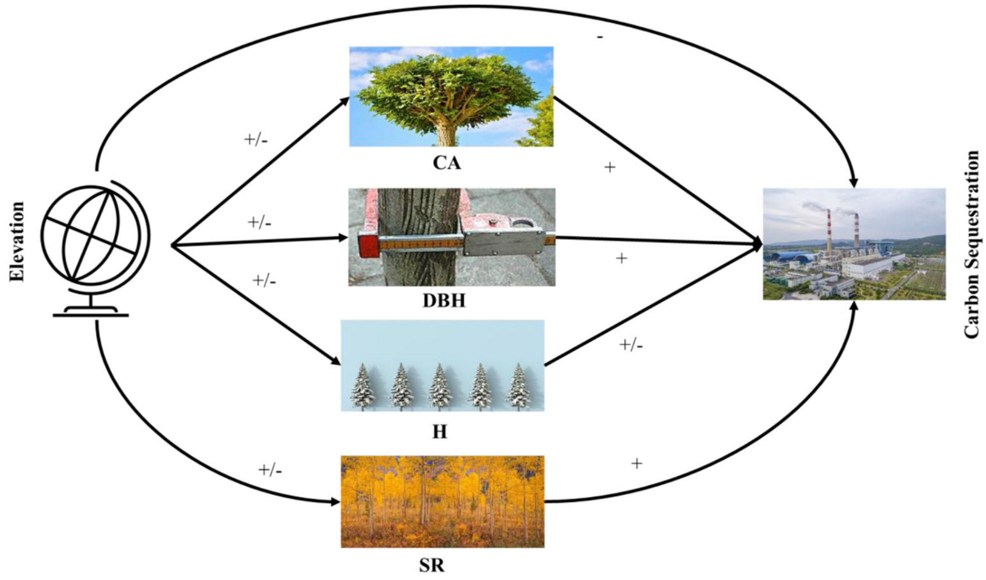


FIGURE 1
Abstract model explaining the direct and indirect effects of the crown area, DBH, height, species richness, and elevation on carbon sequestration. Hypothesized associations among variables are designated by −, +, or −/+, respectively.

SR along the elevation from sea level. We used a structural equation modeling approach to test a hypothesized causal relationship among the response variables using data from 200 plots covering sub-tropical thorn forests, sub-tropical broad-leaved forests, moist temperate mix forests, dry temperate conifer forests, and dry temperate *Pinus gerardiana* (*Chilgoza)* forest plots in Pakistan. The critical research questions are as follows. (i) How does elevation gradients, CA, DBH, H, and SR explain variation in carbon sequestration? We hypothesized that carbon sequestration, CA, DBH, H, and SR decreases along with an increase in elevation (34). (ii) How does carbon sequestration change along the elevation under the influence of explanatory variables CA, DBH, H, and SR? We also hypothesized that tree CA increases carbon sequestration. (iii) How does SR affect carbon sequestration along CA, DBH, H, and elevation? We hypothesized that SR and carbon sequestration have no significant effect on each other. (iv) How does DBH coupled with other explanatory variables, explain variations in carbon sequestration? We hypothesized that DBH has a direct impact on carbon sequestration. (v) How does H impact carbon sequestration along with the CA, DBH, SR, and elevation? We hypothesized there is no effect of H on carbon sequestration (Figure 1).

2. Materials and methodology

2.1. Study area

This study covered a geographical area from “25°18.057” to “35°10108” north latitude and “067°11.298” to “071°56.568” east longitude in the different forests of Pakistan. It has an evaluation range between 50 and 2,700 m above sea level. The different forest types studied are the (i) sub-tropical thorn forests (STTF) of Kirthar National Park, Kirthar mountains, Sindh, (ii) sub-tropical broad-leaved forests (STBLF) of Margalla Hills, lower Himalayas, (iii) moist temperate mixed forests (MTMF) of Murree western Himalayas, (iv) dry temperate coniferous forests (DTCF) of Kumrat, Hindu Kush mountains, and (v) the dry temperate *Pinus gerardiana* (*Chilgoza)* forests (DTPGF) of Shirani, Kohe Sulaiman Mountain, Balochistan (Table 1, Figure 2).

2.2. Data collection using quantitative ecological techniques

A detailed description of tools and techniques is explained in this section.

2.2.1. Estimation of carbon sequestrated by plants annually

Carbon sequestration for each woody tree species was calculated through total DBH and total tree height for all the individuals in each plot (35).

2.2.2. Determining total tree green weight

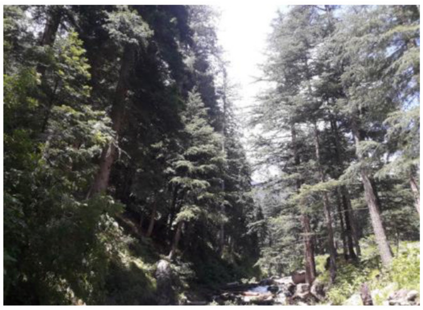
The following algorithmic equation was applied for weighting the carbon sequestration of the woody plant species for 10 years.

$$W_{ag} = 0.15 \times D^2H \qquad (1)$$

where W_{ag} is the above-ground weight of tree species in pounds (lbs), D is the diameter of the tree trunk in inches (for trees with D>1), and H is the height of the tree in feet. The green weight is the live tree weight. First, we calculated the above-ground green weight using the following method (36).

The belowground system weight of a tree is 20% greater than the above-ground weight. We calculated the total green weight of the tree

TABLE 1 Studied forest types along with their elevation range, mean annual temperature and precipitation.

S.No	Forest types	Elevationfrom Sea level (in meters)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Pictorial view
i.	Sub-tropical thorn forest	56–302	33.8	245.3	
ii.	Sub-tropical broad-leaved forest	555–1,117	27.8	1,572.1	
iii.	Moist temperate mix forest	1,249–2,892	17.8	1,596.1	
iv.	Dry temperate conifer forest	1,040–2,566	23.4	1,371.8	

(Continued)

TABLE 1 (Continued)

S.No	Forest types	Elevationfrom Sea level (in meters)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Pictorial view
v.	Dry temperate *Pinus gerardiana* (*Chilgoza)* forest	1,841–2,282	25.9	299.0	

by multiplying the above-ground weight by 1.2.

$$W_{tgw} = 1.2 \times W_{ag} \tag{2}$$

The tree's total average mass is ~72.5% dry matter, and the moisture content of the tree is 27.5%. Hence, we calculate the tree's dry weight by multiplying the total green weight of the tree by 0.725 (37).

$$W_{Dw} = 0.725 \times W_{tgw} \tag{3}$$

The average carbon content in the tree is generally 50% of the total tree volume (16, 37). Thus, we determined the weight of carbon in the tree by multiplying the tree's dry weight by 50% or 0.5.

$$W_c = 0.5 \times W_{Dw} \tag{4}$$

The chemical composition of CO_2 is one molecule of carbon and two molecules of oxygen. The carbon atomic weight is 12.001115 and the oxygen atomic weight is 15.9994. Therefore, the determination of CO2 weight in trees is the ratio of CO2 C+2 × O = 43.999915 C is 43.999915/12.001115 = 3.6663. Thus, we determined the weight of sequestered carbon dioxide in the tree by multiplying the tree's carbon weight by 3.6663, rounded to 3.67 (16, 38).

$$W_{co2} = 3.67 \times W_c\ (v) \tag{5}$$

Tree crown area (CA) was calculated using the formula for the area of an ellipse (Ae):

$$Ae = \pi(0.5x) \times (0.5y) \tag{6}$$

where is the crown length and (y) is the width measured perpendicularly (5, 39).

2.3. Statistical analyses

A structural equation model (SEM) was constructed based on the following conceptual hypothesis: (a) the direct effect of CA, DBH, SR, H, and elevation on the carbon sequestration (b) the direct effect of elevation on CA, DBH, H, and SR; and (c) the indirect effect of elevation, DBH, CA, H, and SR on carbon sequestration. The goodness of fit was calculated using the goodness of fit index (GFI), the chi-squared test, standardized root mean square residual (SRMSR), the comparative fit index (CFI), Akaike's Information Criterion (AIC), and the Bayesian information criterion (BIC) (40). The causal effect amongst the dependent, independent, and mediator variables was tested in the theoretical model while calculating the predicted variable's total effect, direct and indirect, on a response variable. The description of the variable used in the SEM model is represented in Table 2. A VIF test was carried out to check the multicollinearity in data. In our case, the VIF test's importance is around 2.50. Hence, we proceeded with further regression.

The linear regression for each conceptual path was calculated for the complete path measurement of the SEM result, which is shown in Figure 1. However, due to the direct and indirect effects of the other response variables, the results of the bivariate associations may or may not be reliable with the SEM outcome (41–44). Thus, nine series of multiple linear regression models with various response variables were used, for example, (a) the multiple effects of carbon sequestration, CA, H, DBH, and SR; (b) multiple effects of mediator and carbon sequestration; (c) multiple direct and indirect effects of carbon sequestration and response variables. The Hmisc and corrplot packages evaluated the Pearson correlation coefficient matrix.

Before statistical analysis, for normality and linearity, all continuous numerical variables were normalized and standardized and all statistical analysis was done in R 4.0.2 (45).

2.4. Empirical model

We determined the conceptual model with several empirical models. Our generalized model as

$$Y_{co2i} = \alpha + \beta_{CAi} + \lambda_{DBHi} + \delta_{Hi} + \Upsilon_{SRi} + \theta_{Elevi} + \mu_i \tag{7}$$

where CO_2 showed carbon sequestration, CA represents aerial expansion, DBH is the diameter at breast height, H indicates the tree height, and Elev is the elevation of the studied areas, where (i) indicates the number of the plots and α, β, λ, Υ, δ, and θ are the coefficients of the response variables. In contrast, μ is an unobserved variation or error term of the model. We arranged the generalized

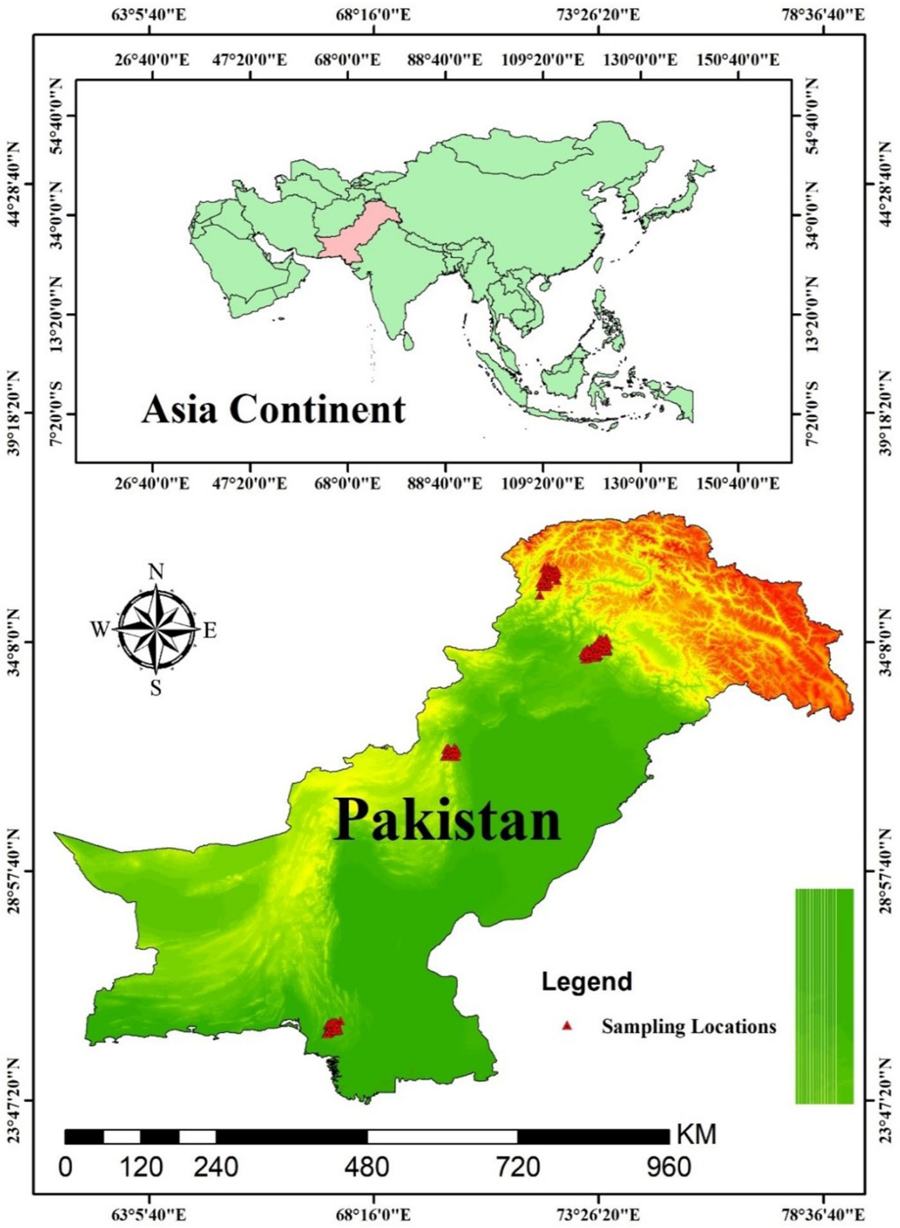


FIGURE 2
GIS-generated map of the studied forests across Pakistan.

model into six parts to find the direct, indirect, and total effects and measure all the below equations simultaneously.

$$Y_{co2i} = \alpha + \beta_{CAi} + \lambda_{DBHi} + \delta_{Hi} \Upsilon_{SRi} + \theta_{Elevi} + \varepsilon i \quad (8)$$

$$Y_{co2i} = \alpha + \beta_{CAi} + \lambda_{DBHi} + \delta_{Hi} + \Upsilon_{SRi} + \varepsilon i \quad (9)$$

$$Y_{co2i} = \alpha + \beta_{CAi} + \lambda_{DBHi} + \Upsilon_{SRi} + \varepsilon i \quad (10)$$

$$Y_{co2i} = \alpha + \beta_{CAi} + \lambda_{DBHi} + \varepsilon i \quad (11)$$

$$Y_{co2i} = \alpha + \beta_{CAi} + \varepsilon i \quad (12)$$

3. Results

Above and belowground CS was found highest in dry temperate conifer forests (DTCF) i.e., 52.67%, followed by moist temperate mix forests (MTMF) sub-tropical broad-leaved forests (STBLF), dry temperate *Pinus gerardiana* (Chilgoza Forest) (DTPGF), and sub-tropical thorn forests (STTF) with 29.99, 11.66, 4.52, and 1.14% contribution, respectively (Table 3). The SEM described 80% of the CS variation i.e., 57, 32, 19, and 17% under the influence of DBH, SR, CA, and H, of the forest trees, respectively (Figure 3). CA had a strong direct effect on the CS (β = 0.90 and P-value < 0.001), followed by the effect of H, DBH and SR with the values of β = 0.13 and P-value = 0.009, β = 0.07 and P-value = 0.039, and β = −0.55 and P-value = 0.001, respectively. The individual direct effect of SR on CS had been negative and significant. At the same time, the separate effects of CA, DBH, and H were positive and significant on CS. The remaining 20% of CS variations were indirectly influenced by elevation. This means that elevation affects CS indirectly through CA, DBH, H, and SR with values of β =

TABLE 2 Description of the independent and dependent variables.

Variables		Description
H	Height of trees	The height was measured through Clinometer (in Feet).
DBH	Diameter at breast height	The diameter at breast height was measured through DBH tap (in inches).
CA	Crown area	The crown Area was measured by following a standard protocol (in meters).
SR	Species richness	The total number of spices per plot was counted (plot size 20 × 20 m).
E	Elevation	The elevation was noted through GPS.
CS	Carbon sequestration	The amount of carbon sequestration was evaluated through the weight of carbon dioxide in the selected plot is equal to 3.67 times the weight of carbon (16).

0.133, followed by $\beta = 0.531$ and P-value < 0.001, and P-value < 0.166, $\beta = 0.007$ and P-value < 0.399, and $\beta = -0.32$ and P-value < 0.001, respectively (Supplementary Table 1). Whereas, the effect of the mediators (CA, DBH, H, and SR) was examined ($\beta = 0.06$ and P-value < 0.001, followed by ($\beta = -0.28$ and P-value < 0.001, $\beta = 0.50.6$ and P-value < 0.000, and $\beta = 0.32$ and P-value < 0.068), respectively, on CS (Supplementary Table 1). The total effect of all response variables jointly on CS was 44% positive and significant (Supplementary Table 1). Pearson correlation analysis shows the relative contribution of CS, elevation, CA, DBH, H, and SR (Figures 4, 5). The correlation analyses evaluated the relationship between dependent and independent variables. The analyses show that elevation had a positive correlation with CS. This means that with an increase in elevation up to a limit, carbon sequestration also increases. The relation between carbon sequestration and the biotic variables were positively correlated with each other except SR, ie., when CA, DBH, and H increase the potential for CS also increases (Figure 5).

The direct theoretical paths are examined with each box to remove the difficulties of the regression path in the model (Supplementary Tables 1, 2). The significant standardized regression coefficients are shown in bold text and the insignificant standardized coefficients are shown in the normal text. Model fit statistic: comparative fit index =0.9000; goodness of fit index = 0.9100; standardized root mean square residual = 0.0032; chi sq. value = 10.07 and P-value = 0.122; AIC = 2215; CIF = 0.885 and GIF = 0.965, respectively in our model. Supplementary Table 1 shows the standardized direct, indirect, and total SR (b4*a1) on various elevation gradients along the regional scale. The indirect effect of CA, DBH, H, and SR significantly affected CS, and the total effect of all variables is also positive and significant (Supplementary Table 1).

The Pearson correlation coefficient measures the strength of the association between the two variables. In the first step of the Pearson correlation, we examined the relationship between two continuous variables and checked the significant effect among all the response variables through the correlation P-value. All the variables had a substantial relationship in our case, so we proceeded with further statistical analysis.

TABLE 3 Primary result of the study in different forest types.

S. no	Site	No of plots	No of trees	Av. No of trees per plot	DBH (%)	Height (%)	Crown area (%)	Species richness (%)	Above and belowground carbon sequestration (%)
1	STTF	40	5,058	126	9.28	7.315	24.52	9.18	1.14
2	STBLF	40	1,923	48	19.15	20.80	23.38	47.95	11.66
3	MTMF	40	5,264	131	27.12	30.00	15.70	31.63	29.99
4	DTCF	40	1,686	42	29.04	30.45	25.18	10.20	52.67
5	DTPGF	40	1,328	33	15.38	11.41	20.20	1.02	4.52

STTF, sub-tropical thorn forests; STBLF, sub-tropical broad-leaved forests; MTMF, moist temperate mix forests; DTCF, dry temperate conifer forests; DTPGF, dry temperate *Pinus gerardiana (Chilgoza)* forests.

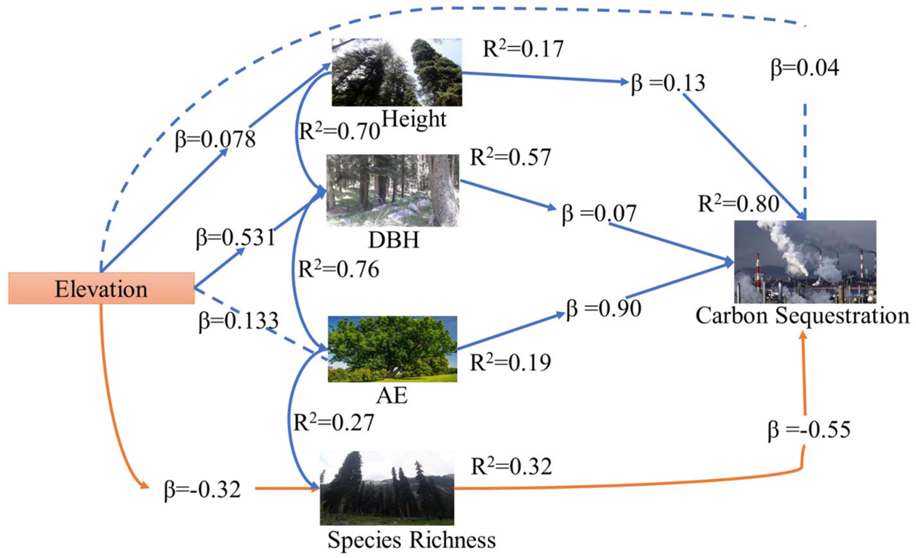


FIGURE 3
SEM linked elevation, CS, CA, DBH, H, and SR in Pakistan's different forests.

4. Discussion

This is the complete estimation of carbon sequestration in different forest types, i.e., sub-tropical thorn forest, sub-tropical broad-leaved forest, moist temperate mix forest, dry temperate conifer forest, and dry temperate *Pinus gerardiana* (Chilgoza) forest along regional-scale elevation gradients in Pakistan. It indicates that CA had a substantial direct effect on carbon sequestration, followed by the impact of H, DBH, elevation, and SR, respectively. We will link our findings on carbon sequestration with previous local, regional, and global studies in this section. The findings of carbon sequestration along elevation gradients with the previous study conducted at Mt Changbai, based on H, DBH, and SR, all decreased significantly with an increase in elevation. As a result, elevation negatively affects carbon sequestration (46, 47). Similar to other previous regional level studies indicate that carbon sequestration, all above-ground and below-ground living biomass were significantly and negatively related to elevation on Mt Changbai, probably due to the temperature drop along the elevation gradient, a comparative shortage of water, and low soil temperature for tree growth on high elevation (48). More importantly, this may be because the carbon dioxide did not reach the higher elevations and the trees absorbed more carbon dioxide at the lower elevations. Many researchers have reported that carbon sequestration decreased with increasing elevation (49). Growth can be restricted by water shortages, exposure, reduced temperatures, transpiration rates, deprived soil quality, and low soil temperatures (50). However, the findings vary with studies from other tropical forests, where the biomass decreases with elevation, resulting in a decline in carbon sequestration (22). Xu et al. (51) studied subtropical forests and found that the canopy density is the major biotic factor in modulating vegetation carbon sequestration in forest ecosystems (51).

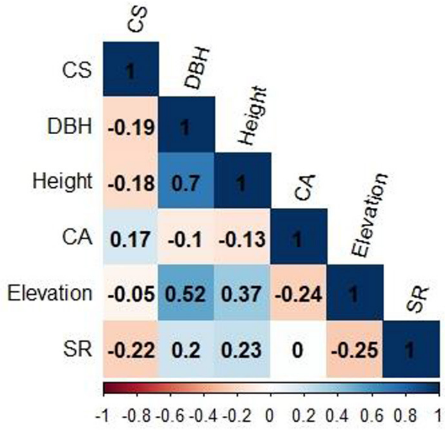


FIGURE 4
Pearson correlation among all predictor variables. CS, Carbon sequestration; DBH, Diameter at breast height; CA, Crown area; SR, Species richness.

Ecologists are interested in the potential functional relation between carbon sequestration and species richness (52). The relationship between CS and species richness is still uncertain and depends on other factors, i.e., elevation and physiographic factors. In the present study, we found that carbon sequestration has a significantly negative relationship with species richness. These findings are supported by other studies (53). Sharma et al. (53), from the Garwal region reported a negative correlation between SR and carbon sequestration. Potter (54) concluded that SR is essential, but not the most critical and appropriate metric in biodiversity and carbon sequestration (54). These findings show similarity with our results, i.e., carbon sequestration has a negative relationship with species richness. However, carbon sequestration was directly affected by tree height and DBH in our study. Another study strongly supports the findings of the current one, i.e., carbon density was directly correlated with tree DBH and height (55).

Similarly, Ali et al. (39), concluded that carbon sequestration in the forest is mainly driven by tree height, DBH, crown area, and stand density in the reserve forests of Pakistan (39). Nevertheless,

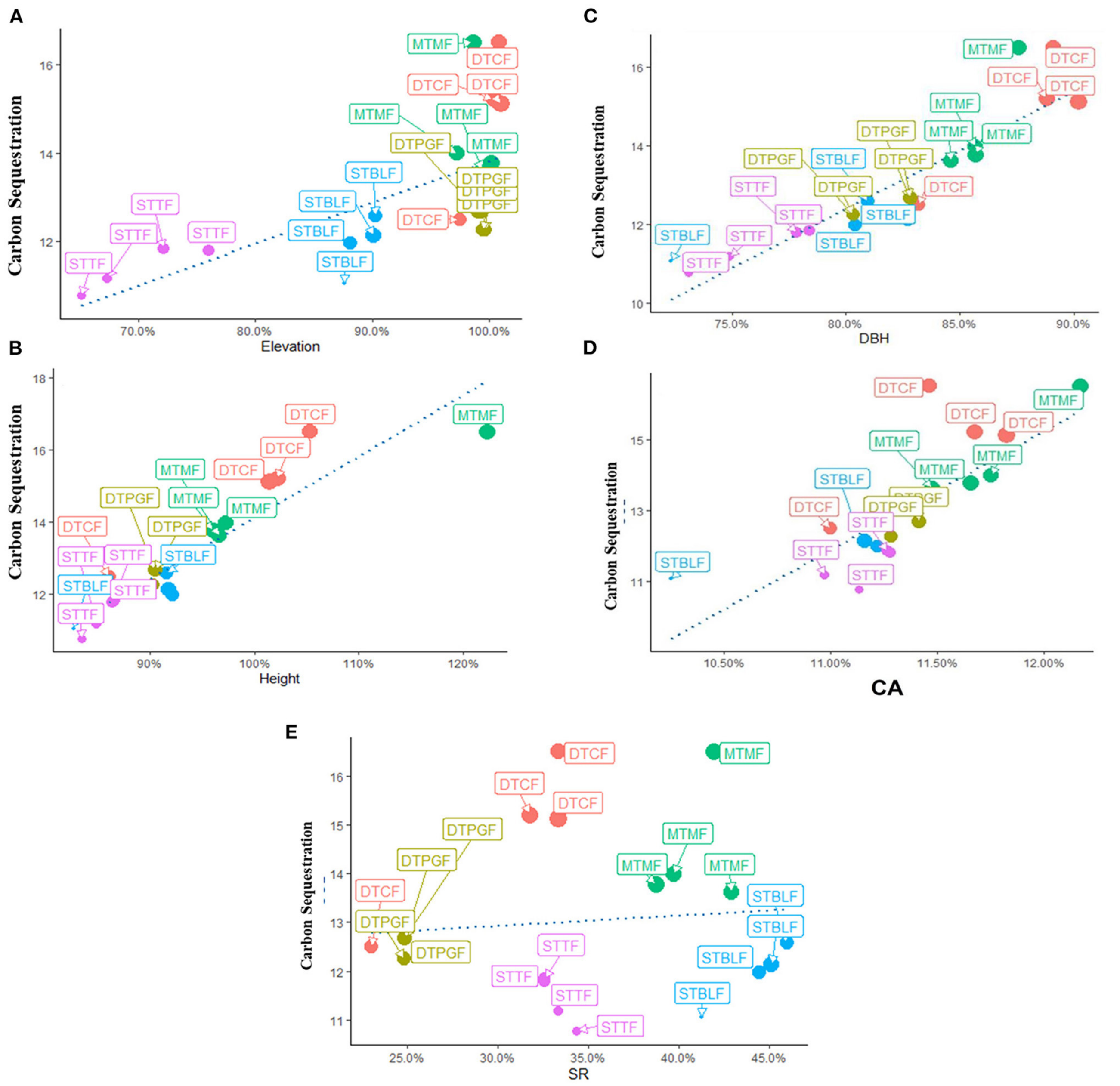


FIGURE 5
Correlation between the dependent and independent variables, i.e., **(A)** Carbon sequestration with elevation, **(B)** Carbon sequestration with H, **(C)** Carbon sequestration with DBH, **(D)** Carbon sequestration with CA, **(E)** Carbon sequestration with SR. STTF, Sub-tropical thorn forests; STBLF, Sub-tropical broad-leaved forests; MTMF, Moist temperate mix forests; DTCF, Dry temperate conifer forests; DTPGF, Dry temperate *Pinus gerardiana* (*Chilgoza)* forests.

other studies reported no correlation between carbon sequestration with species richness and tree height, suggesting that carbon sequestration has no relationship with SR in forest communities. These differences may be due to environmental conditions like precipitation, temperature, etc., which are not present in our study. Despite this, this study directly shows the effect on carbon sequestration by H, DBH, and CA. Similarly, previous studies reveal that tree crown area in complex tropical forests has a strong positive relationship to carbon sequestration across large-scale ecological gradients. These results highlight new perceptions of the tree crown area complementarity mechanism by which the species having a variety of functional traits cause high resource capture and therefore enhance the above-ground biomass (56, 57).

These findings are supported by Afzal and Akhtar (38). According to these results, the DBH and height have a significant relationship with carbon sequestration. Carbon sequestration mainly depends on the H and DBH of the trees (58). These results indicate that CA, DBH, and H, excluding SR, have directly affected carbon sequestration. Tree height and DBH are directly related to carbon sequestration (59). Ali et al. (60) conducted a study in the subtropical forest of eastern China and a skimmia superba broad leaves forest. They revealed that tree height and DBH significantly correlate with carbon sequestration in the subtropical forest (60). Similarly, Moreno et al. (61) conducted a study in southwest Spain and reported similar results to ours that tree height and DBH have significant and positive correlations with tree carbon sequestration capability (61). They concluded that tree height and DBH have a direct relationship with the above-ground biomass and biomass directly related to carbon sequestration (61). Diaz-Balteiro et al. (62) report a similar result from southwest Spain that an increase in tree height and DBH significantly increase carbon sequestration in the forest ecosystems (62). Similarly, the carbon sequestration capacity of tree height and the DBH of the terrestrial vegetation also significantly correlated with each other (63). All these studies strongly support that carbon sequestration mainly depends on tree growth i.e., CA, DBH, H, and SR.

The present study documents the relevant information on live tree CS along an elevation gradient for different forest types. The present study's findings will help us understand the CS pattern in different forest types. This study will also be helpful for researchers to further understand the relationship between CS, CA, DBH, H, and SR along an elevation gradient. Overall, the current research suggested that extensive studies are required to understand the relationship between carbon sequestration and abiotic factors, i.e. soil physicochemical properties, to understand CS's mechanism and natural driver in the wild forest ecosystem. In this study, we did not focus on the abiotic drivers of carbon sequestration, which may have a significant effect on carbon sequestration in the natural forest ecosystems of Pakistan.

5. Conclusions

The present study concluded that a higher above and belowground carbon sequestration potential is found in the dry temperate conifer forest followed by moist temperate forest, sub-tropical broad-leaved forest, dry temperate *Pinus gerardiana* (Chilgoza) forest, and sub-tropical thorn forest. The findings of our research show that CA directly affected carbon sequestration, followed by H, DBH, and SR. There was an insignificant and positive effect recorded of elevation on carbon sequestration. The indirect impact of elevation on carbon sequestration through CA, DBH, H, and SR was recorded positively and significantly. It is also concluded that carbon sequestration is mainly affected by elevation. Furthermore, studies are required to evaluate the relationship between carbon sequestration and multiple biotic and abiotic drivers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

SA contributed to the write-up, fieldwork, and experimental work. SK and AR provided overall supervision, and reviewed and edited the final draft. ZA and ZS helped in data analysis and initial manuscript writing. AU contributed to the field and experimental work. SY and HH provided financial support and edited the final draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Distribution pattern of Crimean–Congo Hemorrhagic Fever in Asia and the Middle East

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Crimean–Congo Hemorrhagic Fever (CCHF) is one of the most important vector-borne diseases of zoonotic potential that can be acquired following the bite of the *Hyalomma* species of ticks. It is a highly prevalent disease in Asia and the Middle East. The risk factors of this disease are contact with infected tissue, blood, patient, or livestock in the acute viremic phase, infected tick bites, or the manual removal of ticks. The disease is clinically described as progressive hemorrhages, fever, and pain in musculature. Biochemical tests reveal elevated levels of creatinine phosphokinase, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase. Clotting time is prolonged in pro-thrombin tests, and pathogenesis is mostly related to the disruption of the epithelium during viral replication and indirectly by secreting cytotoxic molecules. These molecules cause endothelial activation and result in the loss of function. Supportive therapy is given through blood or plasma infusions to treat or manage the patients. According to the most advanced studies, CCHF can be treated by Ribavirin, which is an antiviral drug that shows excellent results in preventing the disease. Health-care staff are more prone to infection. The hemorrhagic phase represents a high risk for accidental exposures. This literature review presents a comprehensive overview of the viral epidemiology, zoonotic perspectives, and significant risk factors of CCHF in various Middle East and Asian countries. Furthermore, the pathophysiology and preventive strategies of CCHF have also been discussed as well as legislation and policies regarding public outreach programs, research, and development aimed at infection prevention and control that are required at a global level.

KEYWORDS

CCHF, prevalence, geographical distribution, pathophysiology, risk factors, zoonosis, ribavirin, prevention

1. Introduction

Ticks and tick-borne diseases have been a threat to humans and animals for many years (1–4). Ticks play an important role as a vector for the transmission of several diseases (5–9). Tick-borne viruses belong to the *Bunyavirales* and *Mononegavirales* orders. These orders contain nine families that cause tick-borne diseases (10). Southeast Asian countries are more vulnerable because of the increasing population and the developing nature of healthcare infrastructure and communities. Crimean-Congo Hemorrhagic Fever (CCHF) is a life-threatening zoonotic disease that affects a vast geographical area (11). CCHF is caused by a virus that belongs to the genus *Nairo* virus, and its family is *Nairoviridae*. It is a negative sense RNA virus containing a segmented genome that is further divided into small (S), medium (M), and large (L) segments as illustrated in Figure 1. The small segment is responsible for the diversity among the viral isolates of different regions (12). The *Hyalomma* tick is responsible for its spread to animals and humans by salivary pathways. CCHF is virulent and potentially hazardous, with the ability

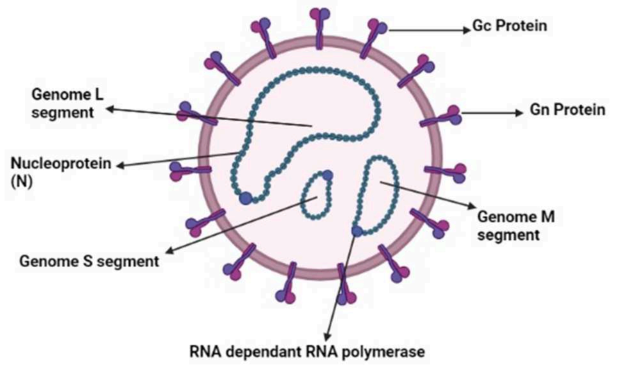

FIGURE 1
Schematic diagram of Nairo virus (Drawn by BioRender app).

to be used as a bioterrorism weapon. CCHF causes 3 to 30% mortality in humans, and becomes disastrous when occurs above the endemic level (13). Tick-bites, animals in the viremic phase, and contact with the blood of an infected patient in the acute phase of infection are all sources of transmission of infection (14). Clinically, the disease is characterized by fever, extensive hemorrhages, and myalgia. Some signs and symptoms, such as hepatomegaly and splenomegaly, are mostly observed in different regions where the disease is prevalent, and these typical signs also vary according to the geographical area and the types of vectors (15). Ribavirin is used as a treatment for CCHF. Effectiveness of the medicine is based only on observational studies, thus it is tentative (16). A few studies suggest that certain beneficial effects are associated with the use of ribavirin (17). In high-risk exposures, treatment is done *via* the use of ribavirin (16). However, there is controversy over the effectiveness of ribavirin as a treatment of CCHF (18).

The fatality rate of the disease warrants the adoption of preventive measures against the CCHF virus (19). In geographical areas where the disease is endemic due to the abundance of tick vectors, one should use protective clothing when these ticks are biologically active and are transmitting the virus to humans (20). The use of repellents and examining the skin and clothing for the removal of ticks could spare tick bite incidents (21). People who live in urban areas are at higher risk of exposing themselves to viremic animals, and, therefore, are advised to wear gloves while handling animal tissue or blood to avoid infection (19). The use of protective aprons or clothing is necessary while treating viremic animal herds. Medical staff around CCHF-infected patients are advised to keep barriers while providing medical care (22). The use of goggles, face shields, gowns, and gloves is mandatory when treating patients or soiled surfaces (23). Health-care staff who experience needle stick injury are administered ribavirin by parenteral route to spare the chances of contracting the viral infection (24).

Further research studies are required to reveal risk factors and transmission patterns of the virus among various hosts (25). The biological roles of previously described vectors need to be discussed in detail while risk factors, such asclimatic changes, reservoir hosts, and other contributory factors, need more investigation. This sort of work requires a consortium that takes on board multidisciplinary professionals for infection prevention and control. Epidemiologists, microbiologists, entomologists, and veterinarians should work together under the theme of One Health to devise ways to curtail the occurrence of the disease. There must be rapid risk communication between these disciplines and ecologists in order to avoid the disease in a particular geographical area. Certain drug trials are needed to develop a drug of choice for CCHF, e.g., heparin and other anti-coagulants can be tested to treat disseminated intravascular coagulation. The prevalence of the CCHF virus in Asian and Middle Eastern countries, its association with humans, pathogenesis, pathophysiology, and treatment strategies of the disease have been discussed in the following review.

2. Viral clades

The genomic clades of CCHF are Clade I, which involves the region of West Africa, Clade II has been found in Central Africa, while Clade III has been found in South and West Africa. Clade IV exists in the Middle East and Asia, while Clade V and VI belong to Europe and Greece, respectively. The asian Clade is further divided into two distinct clades, Asia 1 and Asia 2, respectively (12). This phylogenetic classification of the virus is done based on S segments of the CCHF virus (26).

3. Pattern of distribution in Asian and Middle Eastern countries

CCHF is a highly prevalent disease involving different countries of Asia and other continents (27). The prevalence of CCHF in Asian and Middle Eastern countries is described country-wise in Figure 2.

3.1. Pakistan

CCHF cases in the country are increasing with each passing year (28). In the 1960's, the virus was first identified in ticks infesting local livestock (29). In 1976, CCHF was observed in Pakistan for the first time as a human case. Up to 2010, only 14 cases were reported (30). After 2010, CCHF cases began to increase at a rapid rate. From 2014 to 2020, more than 350 cases of CCHF were confirmed by the National Institute of Health, Islamabad (31). The mortality rate was proposed to be more than 25% (32). Among these cases of CCHF, only 38% were reported from the Balochistan province, 23% were from the Punjab province, 19% from Khyber Pakhtunkhwa, 14% from Sindh, and 6% were reported from the capital city Islamabad (33). In another research study, the prevalence of CCHF was recorded to be 24.7% in Punjab, 16.2% in Sindh, 52.4% in Khyber-Pakhtunkhwa, and 59.3% in Baluchistan (34). The disease was more prevalent in rural areas due to the close interaction of people with the animals. People who live in urban areas tend to be more infected and at a higher rate on the eve of *Eid Ul Adha,* when sacrificial animals are sold and then consumed without proper inspection of animal/animal by-products (35).

Prevalent strains from neighboring countries like Iran and India tend to spread and circulate in Pakistan, and vice versa (36). In 2004, 248 cases were positive for CCHF and, among those cases, only 68% were reported from Baluchistan (Pakistan) and Sistan of Iran (37). In the period from 2004 to 2006, there were annual increases in cases of up to 300 patients. On average, 6% of ticks were positive for harboring the virus, and the Asia 1 and Asia 2 were prevalent strains (38). They are considered clade IV among the genomic sequence.

There are certain risk factors that are playing a crucial role in the spread of CCHF. Rapid climatic changes result in biannual peaks between March to May and August to October. Poor sanitation, unhygienic slaughterhouses, transport of animals within cities, nomadic lifestyle, and lack of trained animals and medical care staff contribute to the spread of CCHF (39).

3.2. China

In 1965, there were reports of hemorrhagic fever in western China. The samples collected from ticks, animals, and humans yielded the CCHF virus upon diagnosis. From 1965 to 1994, 260 farmers were reported to be infected with CCHF. The mortality rate was 80% (40). One imported case was reported in 2013 in the city of Beijing, China. Confirmed reports of the CCHF virus were also received from Xinjiang, Yunnan, and Qinghai provinces in China (41, 42). The CCHF virus was detected among ticks of sheep and camels in the Inner Mongolia region of China (43). From 1951 to 2021, only 447,848 cases were reported to be infected from *bunyavirales* viruses, and CCHFV, along with three other viruses, was reported to cause the most disease burden (44).

3.3. Kazakhstan

In 1944, CCHF was reported for the first time in Kazakhstan (45). Most of the prevalence was reported in Zhambyl and Kyzylorda, regions of the south of Kazakhstan (46). The first reported case of CCHF was in the Turkestan region and was termed Central Asian Fever (47). Later, in 1963 and 1982, cases were reported from Kyzylorda (48) and Zhambyl (49). These regions show a high prevalence of CCHF every year. The mortality rate from CCHF was 14.8% (50) and about 16 cases were reported annually in Kazakhstan (51). In the Zhambyl region, cattle and sheep were tested for the virus and the CCHF virus was found in ~2.4% of sheep and 3.8% of the cattle population (52).

3.4. India

The first case of CCHF was identified in the Gujrat state of India, and it was the result of a nosocomial infection that was related to Pakistan across the border (53). A local survey of livestock revealed that serum and tissue were analyzed to check the prevalence of *H. anatolicum* (54). From 2010 to 2019, 34 outbreaks were reported from the region of Uttar Pradesh, Rajasthan, and Gujarat (55). Eight secondary cases of CCHF were reported out of the 34 cases (56). In another study, CCHF cases were detected in four states of India (57).

3.5. Afghanistan

In 2009, there was an outbreak of CCHF in the region of Herat, Afghanistan. Only 60 positive cases were detected. It was revealed that native breeds of cattle and sheep were harboring high levels of IgG in their blood in the surrounding area, indicative of probable pre-exposure (58). In a study, 51 positive cases of CCHF were detected by ELISA and, of these, 11 patients died. These were butchers and shepherds. Patients with CCHF increased significantly between June to September of the endemic year. This proved that lifestyle and climatic conditions are the risk factors for the spread of the disease (59). Vector ticks of CCHF were identified on the border of Afghanistan and Iran, giving rise to the potential risk of CCHF in humans (60).

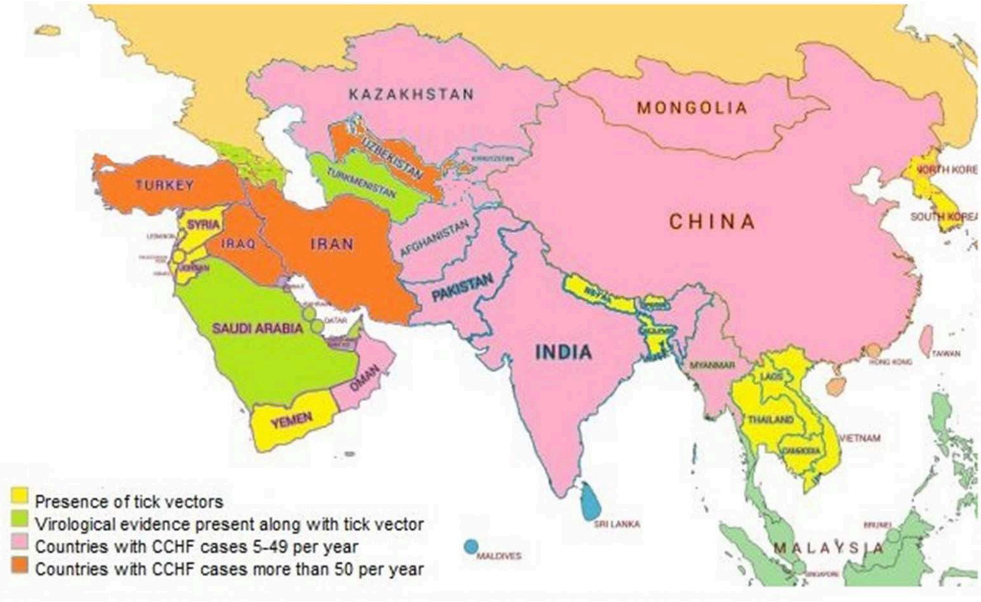

FIGURE 2
Distribution pattern of CCHF in Asian and Middle East countries.

3.6. Malaysia

In a research study, the seroprevalence of CCHF was determined in 2015 within the Orang Asli, a minority population. The titer was too low in them for detection, and it was negative in other populations (61).

3.7. Iran

In the 1970's, the first case of CCHF was reported in Iran, based on the presence of antibodies against CCHF in the serum of cattle, sheep, and humans (62). In Tehran, a sheep abattoir was found to be harboring viral antigens (63). The source of the virus was the ticks of the *Ixodes* genus (64). Human disease reports were reported in 1999 and since then CCHF outbreaks are reported from different regions of the country (65). The mortality rate was 20% in 2000 and it reduced to 6% in 2007 (66). In a study, 203 ticks were checked for the presence of CCHFV and this was absent in the Kerman province although it was an endemic region (67). In 2011, seven butchers from 104 slaughterhouses were detected to be seropositive for CCHF (68).

3.8. United Arab Emirates

In 1979, CCHF was reported for the first time in Dubai, in the Arabian Peninsula, in a health facility outbreak (69) and, thereafter, no other case was observed until 1994 when an epidemic was reported in the United Arab Emirates among abattoir workers (70). A serum investigation was done in native and imported breeds of livestock to determine the antibody titer, and results showed that native breeds were positive for CCHF antibodies while the imported breeds lacked the CCHF antibody titer. This further confirmed the presence of the disease in that region. Livestock import was reported to be the major cause of the disease (71). It also led to 35 confirmed cases of CCHF with clinical pictures (70). In 2010, two cases of CCHF were reported in Dubai (72). Five cases and two deaths were reported between the time frame from 1998 to 2013 (73). *Hyalomma* tick presence was proven in native livestock giving rise to an alarming situation for the risk of spread of CCHF (74, 75).

3.9. Oman

In the mid-1990's, human-infected cases of CCHF were reported from the region of Oman, and the serum analysis of local animals confirmed the presence of the CCHF virus in the region (76). The first case of CCHF was observed in 2011, which was the first case after 15 years (77). In 2014, one death, along with a further 18 human-infected cases, were reported in Oman. Only 16 confirmed cases of CCHF were reported in 2015. The mode of disease transmission was either slaughtered animals or livestock (78). CCHF was transferred *via* occupation in most of the cases occurring in the period from 2011 to 2017 (79). In 2019, from the Northern region of Oman, four patients with CCHF were reported during the festival of *Eid Ul Adha* (80).

3.10. Iraq

Due to war and civil unrest, data collection for CCHF was not efficiently done in Iraq, but certain reports claimed the presence of CCHF in the country (81). Six cases of CCHF were reported between 1989 and 2009. In 2010, 11 cases of CCHF were reported. Three fatal cases were reported in 2018, while 33 cases were reported in 2021, with 13 fatal cases (82). The World Health Organization (WHO) reported 1085 suspected cases of CCHF in 2022, and laboratory confirmation revealed 287 positive cases, with 83 deaths being of suspected CCHF patients and 52 patients confirmed patients of CCHF (18.1% case fatality rate) (83).

3.11. Kuwait

From 1979 to 1982, a serological analysis of 502 patients was done to confirm the disease in two hospitals. Only 18 cases were reported to harbor the disease. According to a research study, only 17 patients who had a close association with livestock had pathognomonic signs of CCHF. About 38% of patients were from rural backgrounds. They were located on the borders of Kuwait so there were chances of imported cases of CCHF (84).

3.12. Egypt

Egypt is a transcontinental country and it showed infections of CCHF in 1978 in many wild and domestic animals. According to a serological study, camels had 8.8% titer while sheep demonstrated 23.1% titer (85). From 1986 to 1987, camel import was the reason for the spread of the disease (86). Antibodies against the CCHF virus were detected again in a 2004 to 2005 serological survey, which confirmed the role of ruminants as a maintenance host (87). Human cases were also observed, mostly in health-care workers and people of rural backgrounds. In 1981 and 2012, a total of four cases were detected, with one death (88, 89).

3.13. Saudi Arabia

In 1990, a case of CCHF was first reported in the country. According to this report, seven people were infected with the virus in Makkah (90). From 1989 to 1990, only 40 workers of slaughterhouses showed suspected signs of CCHF in Makkah, and twelve patients died due to these suspected signs. Imported sheep were considered to be the source of the disease (91). Imported animals and humans who were working on the seaport were also tested for titer of antibodies. Animals along with the staff of the seaport of Jeddah also tested positive for the presence of antibodies against CCHF (92).

3.14. Turkey

It has been observed that Turkey is the hub of CCHF, with reports of about 1,000 confirmed cases per year. In the past few decades, the country was CCHF-free, but with time, Turkey became the lodestone of the disease. It may be possible that there was underreporting or that CCHF was not differentiated from other diseases or was misdiagnosed as some other disease, but it might have been present in the region. Moreover, the ecological and environmental conditions in the country are very favorable for the successful completion of tick life cycles (93). In 2002, the first case of CCHF was reported in patients in the region of the eastern Black Sea in Turkey (93). In 2009, the fatality rate among 500 patients was reported to be 5% (94). This rate was high because initially the disease was misdiagnosed in 66% of patients, as the early symptoms of the disease were not pathognomonic (95). According to a serosurvey conducted in the region of eastern Turkey and Anatolia, specific antibodies against the CCHF virus were about 80%, indicating that the region was most prone to the disease among all regions of Turkey (96). *Hyalomma* ticks were used for viral isolation, and about 20% of them harbored the virus (97). In a study, conducted at a secondary care hospital in Kastamonu, patients with CCHF were evaluated in the period of 2014–2017. A total of 76 suspected cases appeared, and CCHF was confirmed in 46.1% of cases. During those 4 years, the case fatality rate was of only 9.6% (98). A woman suffering from both SARS-CoV-2 and CCHF was treated against both infections and she was lucky enough to beat both viral variants (99).

4. The CCHF virus and its association with humans

The life cycle of the CCHF virus depends upon a vector (ticks) and an amplifying host. There are many species of ticks that are proven to harbor the CCHF virus. The *Ixodes* genus of ticks is the most efficient among all other genera (100), while the *Hyalomma marginatum* is the most efficient vector (101). Other tick species that are reported to transmit the CCHF virus are *H. aegyptium, H. schulzei, H. onatoli, H. dromedarii, H. rufipes, H. excavatum, H. anatolicum, R. sanguineus, R. turanicus, R. annulatus, Ha. punctata, A. variegatum, H. truncatum, H. turanicum, I. ricinus, A. lepidum,* and *H. impeltatum* (74). *Hyalomma marginatum* has certain characteristics that make it a triumphant vector over others. It harbors the virus in its saliva and when the virus reproduces sufficiently in the intestinal tract of ticks then it replicates significantly and tends to spread to the other organs (102). Some organs have a low titer of the virus and few other organs possess a greater number of populations of the virus-like salivary gland and reproductive tract (103). The virus transmits vertically in different stages of life, other than adult stages, i.e., larvae and nymph (104). Female ticks have a tendency to lay thousands of eggs and the transovarian route is also present (105), so even a very low viral titer in females transfers to offspring and also circulates in the environment. These infected ticks also infect the non-infected animals in the vicinity, on the other hand, the infected animals also spread the CCHF virus to ticks that feed blood from infected animals (27). True natural CCHF reservoirs are those ticks that remain infective for a lifetime and never become free from the virus. The *Argasid* genus is not able to transfer virus vertically or horizontally (106).

An amplifying host in the case of the CCHF virus is a vertebrate host, which amplifies the virus and transfers it to humans (107). The CCHF virus tends to multiply at a faster rate in blood and develops a high viral titer within or <14 days. Clinical symptoms are not observed in animals. Large herbivore animals are reported to be seropositive (108). Ostriches are the only birds that harbor the virus, and no other bird species has yet been reported to harbor the virus (109). Birds have a role in outspreading the disease during migration from one area to another as they carry infected ticks in their fur (110). Some small vertebrates, such as hares and hedgehogs, act as a reservoir, maintain the viral load in society, and tend to spread the disease, as illustrated in Figure 3 (111). They are usually infested with larval stages of ticks that transfer the virus in their life stages by vertical mode. On the other hand, large animals are infested with a great number of ticks at the same time (112). The condition can be worsened by the additive effect of horizontal transmission. Favorable conditions such as the hot and arid season help in the molting of tick larvae into the adult tick. Vegetation and humidity also help in the propagation of the ticks. Furthermore, the presence of large and small mammals in a particular area helps in the maintenance of the disease in a particular region. Researchers claim that the CCHF may spread to unaffected regions and countries of the Mediterranean region because they have a feasible environment for ticks (78). Some authors claim that in a few years, Western Europe will be facing a devastating outbreak of the disease because of its climatic adaptation and consistent distribution pattern of *Hyalomma* ticks (113).

5. Transmission and zoonotic impact of CCHF

Humans usually get bitten by an infected tick and develop the infection when they are rearing livestock and handling animals,. In some cases, humans remove ticks from animals and squash them by bare hand, ultimately developing the disease. Tick bites are responsible for 60 to 69% of patients (114). Adult ticks of the *Hyalomma* species complete their lifecycle in spring and summer whenthey feed on amplifying hosts (115). If the accompanied winter was not too harsh, the chances of CCHF cases increase as the tick population does not reduce (116). The ecosystem is also responsible for the spread of the disease in particular areas where a higher number of mammals, large or small, roam, with chances of a spillover to the human population reducing significantly because the virus tends to roam silently in those mammals, causing only sporadic cases of the disease in humans. On the other hand, in 1944 in the era of the Germans, livestock and farm animals were reduced significantly in Crimea, but the cases of CCHF in humans increased (27). Wild hares harbored the ticks rather than livestock and these hares transmitted infections to humans by the bite of *Hyalomma* ticks (27).

Infected tissues and blood of infected animals can also be the source of infection in the human population. An extensive study was done in Turkey from 2002 to 2007 that claimed that out of 1,820 patients, only 62% were in close association with animals (117). After the death of an animal, acidification of the internal environment of the body tends to reduce the viral load, but certain reports showed that tissue and blood infected with the virus can be problematic (108). Only 90% of cases were reported from patients that were professionally in contact with animals or animal tissue, such as farmers, butchers, and people working in slaughterhouses. A serological study revealed that older people have a high titer of anti-CCHF IgG antibodies and so do people who are in close contact with livestock or do not have a good socio-economic status. These

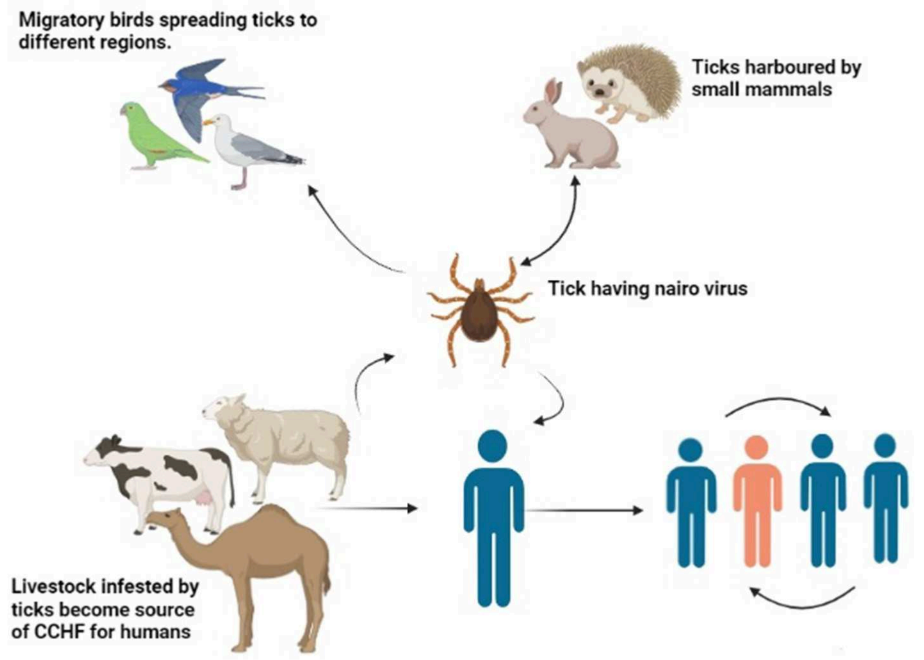

FIGURE 3
Transmission of CCHF virus (Drawn by BioRender app).

people have a higher tendency to be bitten by ticks while handling livestock (118). Most human cases are observed in men because they are more likely to handle livestock and, in Middle Eastern countries where men are mostly performing outdoor jobs, are professionally adapted to animal-associated environments. Sexual transmission has been reported, but there are few such cases, so it has not been prioritized enough (119). In some cases, the virus was transferred vertically and the child showed symptoms of severe hemorrhage and ultimately died (120). On the other hand, infected mothers gave birth to non-infected babies, which suggests that vertical transmission from mother to child does not going happen in all cases of infected mothers (121).

Health workers usually get infected by accidental needlestick injuries while dealing with CCHF-positive patients. In Turkey, nine sites, where 4,869 patients of CCHF were admitted, were examined to check for the prevalence of the disease in health-care staff. Accidental exposure to CCHF was observed in 51 cases, and 25 of those developed CCHF. Among these accidentally exposed medical staff, 16% died due to the exposure. Among these 16%, needlestick injury-associated deaths were about 62.7%. Transmission due to body fluids was about 23.5% (115). Ribavirin was recommended to health workers who were accidentally exposed and, out of 32 patients, 19 were given prophylactic ribavirin and did not develop the disease. On the other hand, eight out of 13 developed clinical diseases and did not receive prophylactic treatment (122). On these bases, ribavirin is recommended as prophylaxis, but in this regard, no document has been formulated yet (122). The hemorrhagic phase of the disease is the riskiest in terms of transmission of the disease to health workers. No case has been reported during the incubation period of the disease (108). The absence of protective measures and the occurrence of needlestick injuries make health workers prone to the disease (108).

6. Pathogenesis of the disease

The exact mechanism by which viruses produce pathogenic effects is not fully known. All the viruses that cause hemorrhagic fever have a common characteristic in which they disable the host's immune function and make it prone to the disease (123). They do so by attacking the antiviral cells. The virus starts to replicate speedily, as shown in Figure 4, and alters the normal functioning of the vascular system and lymphatic organs (124). CCHF pathogenesis is mainly dependent on the infection associated with the epithelium (125). The epithelium is damaged by the continuous replication of viral particles. The second method is indirect damage by the virus, in which the virus releases tissue-toxic factors or produces host-derived soluble factors that result in endothelial activation and loss of proper cellular functions. Damaged endothelium attracts the platelets to aggregate, and the intrinsic pathway of coagulation is activated. It is an early symptom that is obvious and ends as a hemostatic failure.

Cytopenia was associated with haemophagocytosis because it was a consistent finding in half of the patients in Turkey (93). High levels of Type 1 T helper cytokine, such as Tumor Necrosis Factor α, Interferon γ, Interleukin 6, and Interleukin 1, give rise to increased activation of monocytes that lead to haemophagocytic lymphohistiocytosis (126). In this study, it was also revealed that cytokines have roles in the pathogenesis of CCHF (93). It was further confirmed by another study that the level of Type 1 T helper cell cytokines was detected in patients who died and in those who survived. It was noted that the level of all these cytokines was lower in the patients who survived and higher in those who died because of that infection. In grave cases, Interleukin 6 and Tumor Necrosis Factor α levels were higher, along with disseminated intravascular coagulation. While Interleukin10 level was inversely related to them (127).

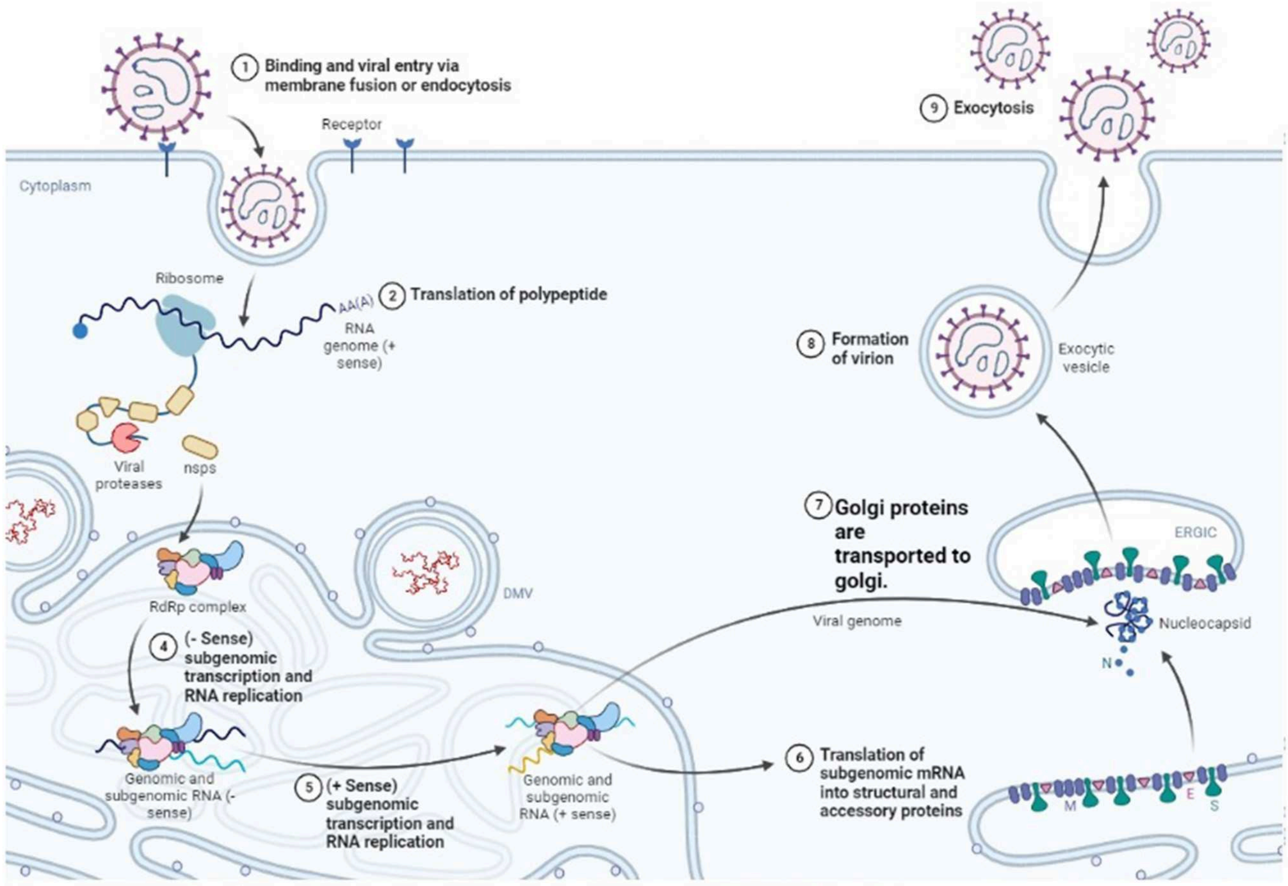

FIGURE 4
Replication cycle of Nairo virus within a host cell (Drawn by BioRender app).

It was noted that cellular pathology was usually associated with the viral division among the cell, but the receptor that allows the virus to move into the cell was not identified yet. Protein domains that are present outside the cell, i.e., GC and GN glycoproteins, play an important role in the binding of the virus to the host cell. The nucleolin present in the host cell also plays a vital role in permitting the virus to cause cellular injury (128). Clathrin-dependent endocytosis enables the virus to enter the host cell (129). Upon entry into the cell, positive strand intermediates are made by viral RNA-dependent RNA polymerase (RdRp) when it interacts with encapsulated genome segments present in the cytoplasm of the host cell. With the help of these positive strands, their complementary negative strands are prepared. A model is drawn in which primer-independent manufacturing of both positive and negative sense genomes is claimed (130).

Microtubules of the host are the main players on which viral internalization, assembly, and egression are dependent (131). PreGN and PreGC, which are immature forms of GN and GC, are synthesized in the rough endoplasmic reticulum and upon their production, the synthesis of viral surface glycoprotein initiates. Both PreGC and PreGn are transferred to the Golgi body as a heterodimer. They are cleaved further, then the process of glycosylation occurs, and the heterodimer is folded and converted into a viral membrane (132). The Golgi body releases mature viruses into the neighboring environment, and this process is known as budding. In this way, the virus replicates and causes different degenerative changes.

7. Pathophysiology of the disease

Humans are the only known host that show clinical symptoms associated with the disease (133). According to a study, the chances of the development of the clinical disease in people harboring the virus were 0.215 to 1 among every five infected people (134). The development of the disease has four phases, which include an incubatory phase, in which replication of the virus happens in the body, the pre-hemorrhagic phase, the hemorrhagic phase, and the convalescent phase (135). The incubation period starts right after the infected tick bite and usually lasts between 3 to 7 days (136). The incubation period depends upon the amount of viral load injected during the bite and the route of exposure (137). Incubation period is shorter when tick directly feeds on blood rather than other transmission routes. Blood and tissue of infected animals take ∼5 days to develop the infection. The human-to-human transmission also takes 5 to 7 days on average (138). The mean duration before presenting patients to the hospital is reported to be 3–5 days in UAE and 5 to 6 days in Turkey (13).

The second phase of the infection is the pre-hemorrhagic phase, in which the person with the infection shows signs of a fever that ranges from 39 to 41°C (139). There is severe headache, dizziness, and muscular pain (140). The patient remains with the fever for 4 to 5 days and then the fever subsides (63). In some cases, additional symptoms, such as diarrhea, vomiting, and nausea, are observed (141). This phase lasts for about 3 days and different parts of the body, such as face and neck, become hyperemic (142). The sclera becomes congested and conjunctivitis is usually observed (143).

The third phase of CCHF is the hemorrhagic phase, which is shorter and tends to be more prominent in terms of clinical symptoms because of hemorrhages. It usually appears on the 3rd to 5th day of the disease (144). No association is generally observed between fever and hemorrhages in patients (133). The shape of hemorrhages ranges from smaller ecchymotic to petechial hemorrhages. Large hematomas are present on the skin and the mucous membranes (145). Clotting time increases in patients suffering from CCHF and a stage comes when blood is thin enough to ooze out of natural orifices, such as the vagina, gingival tissues, and nose (15). Blood is also seen in urine (hematuria) and feces (melena), and bloody discharge also occurs from the uterus (Menometorrhagia) (146). Hemoptysis is also observed in the hemorrhagic phase (147). This phase is often confused with appendicitis if there is only internal bleeding and there is no sign of external bleeding (148). Persistent pain was thought to be caused due to inflammation in the appendix, but with further investigation, it was claimed that there were internal hemorrhages and bleeding in the cecum, and internal and external oblique muscle with no pathology related to the appendix (149). Hepatomegaly and splenomegaly were also observed in some patients suffering from CCHF, but it was not a consistent finding (150). These were the clinical features of CCHF patients who either recovered from that phase or died due to extensive bleeding and hemorrhages (151).

The last phase is the convalescent phase for those who survived the infection, and it starts about 10 to 20 days after the infection (152). Patients recovering from CCHF have a weak pulse, often accompanied by tachycardia, partial or complete alopecia, dyspnea, polyneuritis, xerostomia, deafness, memory loss, blindness, or weak eyesight (32). Some patients may have bradycardia and a drop in blood pressure (153).

8. Lab investigations in CCHF patients

The basic marker in the diagnosis of CCHF lab reports is a decreased level of platelets and leukocytes (Figure 5). Enzymes, such as aspartate aminotransferase, alanine aminotransaminase, creatinine phosphokinase, and lactate dehydrogenase, tend to increase. Prolonged clotting time is checked with a prothrombin test and an activated partial thromboplastin test. Fibrinogen is reduced, which tends to make a meshwork to bind platelets and protein to make a clot. An increase in the degradation products of fibrin could be observed (154). Within 5 to 9 days, the surviving patients' lab results tend to become normal.

9. Treatment

The treatment strategy of CCHF involves two aspects, one is to give symptomatic treatment to cover up the deficiencies that occur because of the extensive loss of blood cells, such as blood transfusion, platelets, or plasma is given to the patients (115).

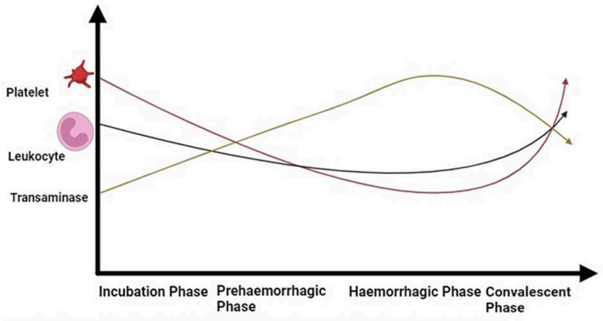

FIGURE 5
Dynamic of blood cells during different phases of CCHF in patients (Drawn by BioRender app).

Hypovolemic patients are given electrolytes. Secondary infections are also addressed as there is immune suppression and the person becomes prone to other diseases (155). Any ongoing infection is also treated. For example, in some cases, malaria occurs along with CCHF in some patients (156).

Ribavirin is also used as treatment and, in some instances, it is given as prophylaxis. The WHO recommends the dose rate of ribavirin for patients. Firstly, it is recommended to give two grams of ribavirin per oral route. Then, until the 4th day, it should be given after an interval of 6 h and by a dose rate of 1 g. From day 5 to 10, it should be increased up to 500 g, with the same hour interval. Parental use has poor bioavailability, but it is used in some instances. The dose of ribavirin recommended is 17 mg/kg, which should not exceed 1 g until the 4th day and with an interval of 6 h. Then it is reduced to half (8 mg per kg) from day 5th to the 10th day. Ribavirin is considered safe with limited side effects and is used efficiently for the treatment of CCHF. Favipiravir, an antiviral drug, was tested in mice and reported to be better as compared to ribavirin, displaying results even when it was given to patients who exhibited symptoms of CCHF (157). However, due to a lack of enough evidence, it cannot be recommended for daily use for the treatment of CCHF.

10. Future perspectives

The exact manner of pathogenesis other than replication needs to be discovered. Research is needed to reveal the various mechanisms of disease production. Once identified, they can be used for the development of certain drugs or candidate vaccine virus that can block the pathway of development of infection by the virus. The impact of the enzootic environment needs to be examined in further detail. The transmission cycle of the CCHF virus and its vectors needs to be analyzed so that there may be a step from where we can break their cycle, ultimately resulting in the downregulation of the disease in a specific region. The field of pharmacology needs to excel to produce such antiviral drugs that can reduce the number of viruses in patients, either by killing the viruses or blocking their replication pathways. Death by CCHF is mostly attributed to disseminated intravascular coagulation. Anticoagulation factors, such as heparin and certain oxalates, can be tested to prevent Disseminated Intravascular Coagulation in patients and clinical symptoms of the disease. The mechanism by which the virus develops Disseminated Intravascular Coagulation in patients needs to be studied, and further studies on CCHF will result in the discovery of thisexact mechanism and how bacterial sepsis develops along with Disseminated Intravascular Coagulation. This understanding will lead to the development of drug molecules that will help to eliminate the disease around the globe. For this, all medical fields, including pharmacologists, pathologists, parasitologists, microbiologists, and clinicians have to work hand in hand to 1 day conquer the disease.

11. Conclusion

The presence of ticks and a suitable environment make CCHF an alarming disease in Asian and Middle Eastern countries. The prevalence of CCHF is noted to be increased per annum almost in all countries, including Pakistan, India, China, Iran, Kazakhstan, Egypt, Iraq, U.A.E, Saudi Arabia, and Turkey. Humans in close contact with livestock are at a greater risk than those in urban areas. Virus pathogenesis is attributed either by directly damaging cells by proliferation or indirectly by releasing cytotoxic compounds. Further investigations are required to discover the exact mechanism of the disease and to provide better healthcare to patients in different clinical phases of CCHF. To prevent zoonosis and transfer to medical health workers, certain measures should be taken to avoid the infection. Drugs with higher efficiency can be prepared once hidden mechanisms of disease are known. Prevention can be the key to success. Treatment can be undertaken using ribavirin and the medication can be given as prophylaxis. Vaccination development needs to be considered in the future for the advancement of better immunity in individuals.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Epidemiological investigation of norovirus infections in Punjab, Pakistan, through the One Health approach

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Introduction: Norovirus, mainly associated with acute gastroenteritis, is very contagious and can affect a vast range of species ranging from cattle, pigs, dogs, mice, cats, sheep, and lions to humans. It is a foodborne pathogen that mainly transmits through the fecal–oral route.

Methods: This is the first-ever study conducted in Lahore and Sheikhupura districts of Punjab, Pakistan, to investigate noroviruses through the One Health approach. From January 2020 to September 2021, 200 fecal samples were collected from clinical cases of hospitalized patients and 200 fecal samples from sick animals at veterinary hospitals and local farms. In addition, 500 food and beverage samples were collected from street vendors and retail stores. A predesigned questionnaire was used to assess the risk factors and clinical characteristics of sick people and animals.

Results and discussion: Overall, 14% of the human clinical samples were positive by RT-PCR for genogroup GII. All bovine samples were negative. Food and beverage samples were tested in pools, resulting in sugarcane juice samples positive for genogroup GII. Previous contact with acute gastroenteritis patients, sex, and presence of vomiting were found to be significant risk factors ($p \leq 0.05$). The substantial number of diarrhea cases associated with noroviruses calls for additional studies to investigate the epidemiology and transmission and to improve surveillance.

KEYWORDS

diarrhea, acute gastroenteritis, One Health, noroviruses, genogroup II and III, Pakistan

1. Introduction

Noroviruses are single-stranded, non-enveloped, and non-segmented RNA viruses having icosahedral symmetry belonging to the family Caliciviridae. Based on the VP1 protein region, they are divided into 10 genogroups ranging from GI to GX (1). Noroviruses are the major cause of acute gastroenteritis among people of all ages but most commonly among children of <5 years of age (2). Globally, acute viral gastroenteritis is a major public health problem, and noroviruses cause an economic burden of $4.2 billion in direct health system outlays and $60.3 billion in societal costs per annum (3, 4). NoV is highly contagious, and a low dose of the virus is enough to cause infection, i.e., only 10–18 virus particles (5). Norovirus infects a wide range of hosts, including livestock animals, pets, marine mammals, rodents, and humans (6). Human infections are mostly caused by GI, GII, GIV, GVIII, and GIX, although GII is the most frequently detected genogroup in humans worldwide including Pakistan (7, 8), and the GIII genogroup is found in bovines (9). Animal noroviruses found in pigs, dogs, and cats are closely related to human strains and cluster in GII and GIV genogroups (10). Thus far, there is no evidence of zoonosis in noroviruses, although some serological evidence has been reported in some previous studies (6). Since the identification of noroviruses in animals and their relatedness to human strains, the question of crossing the species barrier has been raised. This was demonstrated by a study in which gnotobiotic pigs were infected with a human NoV strain, which raises the question of zoonosis (11). Applying the One Health approach for a multidisciplinary investigation is gaining insight to study the disease ecology of noroviruses (12). In humans, noroviruses can cause symptoms of severe vomiting, nausea, watery diarrhea, abdominal cramps, general malaise, and fever (13). The fecal–oral route is the main route of transmission, while other modes of transmission are person-to-person direct contact, consumption of contaminated food and water, exposure to contaminated environmental surfaces, and airborne vomit droplets. Factors such as the high NoV prevalence in the community, the shedding of infectious virus particles from asymptomatic individuals, and the high stability of the virus in the environment contributed to high levels of transmission (14). Bovine norovirus (BNoV) is classified into genogroup III (9), which is frequently detected in calf diarrhea cases and is a persistent threat to the livestock industry (15, 16). It causes mild diarrhea and transient anorexia in calves (17). Calf diarrhea is a substantial cause of economic losses in the livestock industry (18). The prevalence of bovine noroviruses ranges from 7.5 to 49.6% among different countries (19). Noroviruses are foodborne pathogens, and in Pakistan, foodborne illness investigations are focused traditionally on bacterial pathogens (20). Vegetables and fruits, as well as fast foods, baked products, and salads, present a potential health hazard due to unhygienic food handling practices (21–23). Previous studies conducted in Pakistan detected 16.1% and 19.5% prevalence for NoV in humans; although no studies have been conducted to investigate noroviruses in animals and foods and beverages (8, 24). Using the One Health principles to understand the ecology of noroviruses in Pakistan, the objectives of this study were to describe the presence of norovirus in people, animals, and food sources and the associated risk factors.

2. Materials and methods

2.1. Study design and sample collection

The study design was cross-sectional with a convenience sampling method. The study was approved by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore (059/IRC/BMR and DR/072). The study areas were the districts of Lahore and Sheikhupura in the Punjab Province. The study was conducted from January 2020 to September 2021.

Human stool samples were collected from children under 5 years of age from the Sheik Zaid and Children's hospitals of Lahore and two government Tehsil hospitals of Sheikhupura. Children included in the study were hospitalized for acute gastroenteritis as the primary illness and had watery diarrhea for fewer than 7 days with a frequency of three times or more per day along with dehydration (8). All the children were on oral or intravenous rehydration therapy. After informed written consent from the parents/guardians, up to 200 whole stool samples were collected from the diapers in a sterile container by applying a sterile plastic sheet and were stored at −80°C until further processing. A questionnaire was administered to the parents/guardians at the time of sample collection to collect demographic, clinical, and risk factors data.

Animal stool samples were collected from diarrheic calves presented for treatment at veterinary outdoor clinics and by visiting the local farms of Lahore and Sheikhupura which were conveniently accessible. The inclusion criteria were loose stool at the time of the visit and <1 year of age. Samples were collected from the rectum in a sterile container and were stored at −80°C until further processing. Enrollment and sampling continued until 200 stool samples were collected. Data collected included age, sex, fever status, duration and episodes of diarrhea, presence of quarantine area if any for disease animals, previous illness, and practice of visiting veterinary hospitals during the diarrhea illness.

Food and beverage samples were collected from different zones of Lahore district. Target food types including meat, vegetables, and fruits were collected from restaurants, local vendors, and retail shops. Sampling sites were selected based on convenience sampling by their location area and enrolled based on their willingness to provide food samples. Samples were collected using swabs following standard procedures for norovirus testing (25). Sampling continued until 500 samples of meat and 500 samples of other food items such as fast food, fresh juices, and salads were collected. For a given sample type, only one sample per vendor was collected. For meat, the swab was moistened in phosphate-buffered saline (PBS) and used for swabbing a defined area methodically in a horizontal, vertical, and diagonal direction. Whole juice samples and whole salads with a ratio of 1:10 for PBS were collected in a sterile container. All the food and beverage samples were tested in pools. Five samples of the same type were included in one pool.

2.1.1. RNA Extraction and cDNA Synthesis

Fecal suspensions of each sample were prepared by diluting feces 1:10 (w/v) in ProSpecT^{TM} sample diluent. These sample suspensions were mixed for 30 s and centrifuged at 8,000 rpm for 10 min at 4°C (8). Food and beverage samples were dipped and pressed in a sterile container having 9 ml of 1 × phosphate-buffered saline (PBS). RNA was extracted by the TRIzol method by TRIzol^{TM} LS Reagent (Invitrogen^{TM}). cDNA was synthesized at 42°C for 60 min and 70°C for 5 min with eluted RNA using the Thermo scientific^{TM} RevertAid First Strand cDNA Synthesis Kit in a Thermal cycler (T100 Thermal Cycler, Bio-Rad, USA).

2.1.2. Polymerase chain reaction (PCR)

Extracted DNA was amplified using Thermo scientific^{TM} DreamTaq^{TM} Green PCR Master Mix kit and specific primers set G2SKF CNTGGGAGGGCGATCGCAA and G2SKR CCRCCNGCATRHCCRTTRTACAT of product size 346 bp for genogroup GII (24) and for animals CBECu-F: AGTTAYTTTTCCTTYTAYGGBGA and CBECu-R: AGTGTCTCTGTCAGTCATCTTCAT primers set of product size 532 bp for GIII were used (16). For food and beverage samples, both primer sets of genogroup GII and GIII were used to identify the challenge virus. For the GII genogroup, PCR was optimized at 35 cycles using the following thermocycling conditions: 94°C for 30 s, 59°C for 45 s, and 72°C for 60 s followed by a final extension for 10 min at 72°C (24). For the GIII genogroup, PCR was optimized at 35 cycles using the following thermocycling conditions: 94°C for 3 min, 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s followed by a final extension for 10 min at 72°C (16). PCR was carried out in a Thermal cycler (T100 Thermal Cycler, Bio-Rad, USA). PCR products were amplified at 2% agarose gel and were observed under UV light. All the samples were run with positive controls obtained from the National Institute of Health (NIH), Pakistan, and the Institute of Animal Husbandry and Veterinary Science, Henan Academy of Agriculture Science, China.

2.2. Statistical analysis

All the samples were classified as positive and negative based on the PCR results. For each sample type, results were first analyzed to describe the proportion positive overall and by the district. The statistical association between sample positivity and demographic characteristics, clinical signs, and risk factors was evaluated using the chi-square test and Fisher's exact test with a 95% confidence interval (CI). A p-value of ≤ 0.05 was considered statistically significant. The odds ratio (OR) was used to quantify the strength of the association between each risk factor and sample positivity. Episodes of diarrhea and vomiting and the duration of symptoms data collected in continuous numbers were classified for statistical analysis. All analyses were conducted using Statistical Package for Social Sciences (SPSS ver. 20.0).

2.3. Phylogenetic analysis

From the humans' positive samples, three random samples (GIIUVAS1, 2, and 3) were selected for the phylogenetic analysis of evolutionary analysis by the maximum likelihood method. PCR products were submitted for sequencing to a commercial company (Advanced Biosystems, Lahore). The accuracy of data was confirmed by bi-directional sequencing. Previously published sequences of norovirus genogroup GII and genogroup GIII from humans, animals, and water obtained from the National Center for Biotechnology Information (NCBI) were used as reference sequences. The study sequences were aligned and compared with reference sequences using ClustalW. Unique nucleotide sequences generated from this study were deposited in GenBank under accession numbers ON596245, ON596246, and ON596247.

3. Results

3.1. Humans

A detailed description of the results is provided in Table 1. Among 200 participants enrolled in the study, 61% were in the age group of >16 months, while 39% were in <33 months of the age group; 60% of the samples were collected from male participants, while 40% were from female participants. Laboratory analysis of stool samples yielded a prevalence of 14% (28/200) overall, 15% (15/100) in Lahore district, and 13% (13/100) in Sheikhupura. Norovirus positive proportion was 13.9% (17/122) in children <16 months of age and 14.1% (11/78) in the older ones with a p-value of 0.9. Norovirus positivity was significantly greater in female patients (19.8%, 16/81) than in male patients (10.1%, 12/119) (p-value = 0.05). The disease positivity was more, 15.5% (13/84), in children with 7–12 episodes of diarrhea than in children with 1–6 episodes, 12.9% (15/116), per day (p-value = 0.6). Vomiting was present in 16.7% (19/114) of participants, while in 10.5% (09/86) of samples, vomiting was absent. From the Sheikhupura district, vomiting in children was found significantly associated with the disease (p-value = 0.03) with a percentage of 21.4% (09/42). The percentage of positive and negative cases with the presence or absence of abdominal cramps was almost the same, i.e., 13.9% (17/122) in cases with abdominal cramps and 14.1% (11/78) in cases without abdominal cramps. Fever was not common in the positive cases, and most of the cases were positive for the noroviruses without fever, i.e., 19.1% (13/68) without fever and 11.4% (15/132) with fever were positive. Most of the cases, i.e., 16.7% (15/90), in the study were evaluated for a moderate level of dehydration by the physician. Disease positivity was more in children who had symptoms from the last 4–6 days, i.e., 15.3% (09/59), than in the 1–3 days, i.e., 13.5% (19/141). The percentage of positive cases was greater in children who ate any type of meat, 14.4% (24/166), compared with no meat, 11.8 (04/34), but the difference was not statistically significant (p-value = 0.6). Previous contact with acute gastroenteritis patients was found significantly associated with norovirus infection (p-value = 0.001).

3.2. Bovines

A total of 100 samples were collected from the eight clinics and 100 from five farms. Approximately 71% (142/200) of samples were collected from calves of <6 months of age. Sex distribution was 42% (84/200) male patients and 58% (116/200) female patients. At the time of sample collection, 83% of the calves had a fever, 49% were off the feed, and 67% had a history of diarrhea. The frequency of loose stools was 3–4 times/day for 81% and 6–8 times/day for the rest. The data obtained from calves enrolled from the farms showed that only 9% of owners responded "Yes," that is, they visit the hospital during diarrhea. Only 10% of the owners reported having quarantine areas for sick animals. All 200 bovine fecal samples were negative for genogroup GIII by PCR.

TABLE 1 Association of demographic characteristics, clinical signs, and potential risk factors with PCR results in children from Sheikhupura and Lahore districts.

Demographic characteristics	Lahore			Sheikhupura			Total		
	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)
Sex									
Male	08/67 (11.9%)	0.2	0.5 (0.1–1.5)	04/52 (7.7%)	0.1	0.3 (0.1–1.2)	12/119 (10.1%)	0.05	0.4 (0.2–1.0)
Female	07/33 (21.2%)			09/48 (18.8%)			16/81 (19.8%)		
Age (months)									
≤16	10/61 (16.4%)	0.6	1.3 (0.4–4.2)	07/61 (11.5%)	0.5	0.7 (0.2–2.3)	17/122 (13.9%)	0.9	0.9 (0.4–2.2)
≥17	05/39 (12.8%)			06/39 (15.4%)			11/78 (14.1%)		
Clinical signs									
Episodes of diarrhea									
1–6	05/42 (11.9%)	0.4	0.6 (0.2–2.0)	10/74 (13.5%)	0.7	1.1 (0.3–4.7)	15/116 (12.9%)	0.6	0.8 (0.3–1.8)
7–12	10/58 (17.2%)			03/26 (11.5%)			13/84 (15.5%)		
Vomiting									
Yes	10/72 (13.9%)	0.6	0.7 (0.2–2.4)	09/42 (21.4%)	0.03	3.6 (1.0–12.9)	19/114 (16.7%)	0.2	1.7 (0.7–3.9)
No	05/28 (17.9%)			04/58 (6.9%)			09/86 (10.5%)		
Abdominal cramps									
Yes	11/67 (16.4%)	0.5	1.4 (0.4–4.8)	06/55 (10.9%)	0.4	0.6 (0.2–2.1)	17/122 (13.9%)	0.9	0.9 (0.4–2.3)
No	04/33 (12.1%)			07/45 (15.6%)			11/78 (14.1%)		
Fever									
Yes	08/73 (10.9%)	0.06	0.3 (0.1–1.09)	07/59 (11.9%)	0.6	0.7 (0.2–2.5)	15/132 (11.4%)	0.1	0.5 (0.2–1.2)
No	07/27 (25.9%)			06/41 (14.6%)			13/68 (19.1%)		
Dehydration									
Mild	05/31 (16.1%)	0.8	1.1 (0.3–3.6)	08/79 (10.1%)	0.09	0.3 (0.1–1.2)	13/110 (11.8%)	0.3	0.6 (0.3–1.4)
Moderate	10/69 (14.5%)			05/21 (23.8%)			15/90 (16.7%)		
Duration of symptoms (days)									

(Continued)

TABLE 1 (Continued)

Demographic characteristics	Lahore			Sheikhupura			Total		
	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)
1–3	11/73 (15.1%)	0.9	1.02 (0.2–3.5)	08/68 (11.8%)	0.5	0.7 (0.2–2.4)	19/141 (13.5%)	0.7	0.8 (0.3–2.0)
4–6	04/27 (14.8%)			05/32 (15.6%)			09/59 (15.3%)		
Risk factors									
Playing area									
Home	14/85 (16.5%)	0.3	2.7 (0.3–22.7)	09/69 (13.1%)	0.9	1.0 (0.2–3.5)	23/154 (14.9%)	0.4	1.4 (0.5–4.0)
Outside	01/15 (6.7%)			04/31 (12.9%)			05/46 (10.9%)		
Animal contact									
Yes	02/18 (11.1%)	0.6	0.6 (0.1–3.2)	02/37 (5.4%)	0.08	0.2 (0.05–1.2)	04/55 (7.2%)	0.09	0.3 (0.1–1.1)
No	13/82 (15.9%)			11/63 (17.5%)			24/145 (16.5%)		
Hands washing									
Yes	14/94 (14.9%)	0.9	0.8 (0.09–8.06)	11/63 (17.5%)	0.08	3.7 (0.7–17.7)	25/157 (15.9%)	0.1	2.5 (0.7–8.8)
No	01/06 (16.7%)			02/37 (5.4%)			03/43 (6.9%)		
Milk									
Animal source	08/37 (21.6%)	0.1	2.2 (0.7–6.6)	04/42 (9.5%)	0.3	0.5 (0.1–2.0)	12/79 (15.2%)	0.6	1.1 (0.5–2.6)
Breast feeding	07/63 (11.1%)			09/58 (15.5%)			16/121 (13.2%)		
Meat									
Yes	11/74 (14.9%)	0.9	0.9 (0.2–3.3)	13/92 (14.1%)	0.2	0.8 (0.7–0.9)	24/166 (14.45%)	0.6	1.2 (0.4–3.9)
No	04/26 (15.3%)			00/08 (0%)			04/34 (11.8%)		
Food source									

(Continued)

TABLE 1 (Continued)

Demographic characteristics	Lahore			Sheikhupura			Total		
	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)	Proportion positive	*P*-value	Odds ratio (95% CI)
Restaurant	06/39 (15.3%)	0.9	0.9 (0.3–2.9)	13/85 (15.3%)	0.1	1.1 (1.07–1.2)	19/124 (15.3%)	0.4	0.7 (0.3–1.7)
Home made	09/61 (14.8%)			00/15 (0%)			09/76 (11.8%)		
Type of juice									
Fresh	05/45 (11.1%)	0.3	0.5 (0.1–1.7)	07/45 (15.6%)	0.4	1.5 (0.4–4.8)	12/90 (13.3%)	0.8	0.9 (0.4–2.0)
Packed	10/55 (18.2%)			06/55 (10.9%)			16/110 (14.5%)		
Washing of fruits and vegetables									
Yes	14/89 (15.7%)	0.5	1.8 (0.215.7)	10/68 (14.7%)	0.4	1.6 (0.4–6.5)	24/157 (15.3%)	0.3	1.7 (0.5–5.3)
No	01/11 (9.1%)			03/32 (9.3%)			04/43 (9.3%)		
Source of drinking water									
Untreated	05/28 (17.9%)	0.6	1.3 (0.4–4.3)	09/79 (11.4%)	0.3	0.5 (0.1–1.9)	14/107 (13.1%)	0.6	0.8 (0.3–1.8)
Treated	10/72 (13.9%)			04/21 (19.1%)			14/93 (15.1%)		
Hospital visit during illness									
Yes	15/86 (17.4%)	0.09	0.8 (0.7–0.9)	12/97 (12.4%)	0.2	0.2 (0.02–3.3)	27/183 (14.8%)	0.3	2.7 (0.3–21.7)
No	00/14 (0%)			01/03 (33.3%)			01/17 (5.9%)		
Previous contact with acute gastroenteritis patient									
Yes	08/30 (26.7%)	0.03	3.2 (1.06–10.07)	08/31 (25.8%)	0.01	4.4 (1.3–15)	16/61 (26.2%)	0.001	3.7 (1.6–8.5)
No	07/70 (10%)			05/69 (7.2%)			12/13 (92.3%)		
Previous illness									
Yes	11/74 (14.9%)	0.9	0.9 (0.2–3.3)	06/43 (13.9%)	0.8	1.1 (0.3–3.7)	17/117 (14.5%)	0.7	1.1 (0.4–2.5)
No	04/26 (15.4%)			07/57 (12.3%)			11/83 (13.3%)		

3.3. Food and beverages

Meat samples collected included mutton (n = 150), beef (n = 150), and poultry (n = 200). Whole food items collected from fast food stores included paratha rolls (n = 3), shawarma (n = 15), pizza (n = 12), frozen nuggets (n = 50), and frozen chicken wings (n = 20). Fresh juices included sugarcane (n = 45), black currant (n = 27), strawberry (n = 18), watermelon (n = 40), mango (n = 36), lemon water (n = 27), black plum (n = 19), labun (n = 36), plum (n = 34), and tamarind water (n = 18). Vegetables from salads included onion (n = 28), tomato (n = 14), garlic (n = 11), cucumber (n = 21), and cabbage (n = 26). From the 200 food and beverage pools tested, two pools were PCR positive for NoV genogroup GII. All other pools were negative for both GII and GIII genogroups. The two positive pools consisted of sugarcane juice and each sugarcane juice pool contained samples from five different vendors. The positive pool results indicated that at least one individual sample represented in each of the two pools was positive. Due to budget constraints, the individual samples from the positive pools were not tested. Pool positivity calculated from all the samples was 1% (2/200) and for juice, it was 3.33% (2/60).

3.4. Phylogenetic analysis

The evolutionary history was inferred using the maximum likelihood method and the Tamura-Nei model (26). The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed (27). Branches corresponding to partitions reproduced in <50% of bootstrap replicates collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test of 100 replicates is shown next to the branches (Figure 1) (27). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log-likelihood value. This analysis involved 18 nucleotide sequences. There were a total of 306 positions in the final dataset. Evolutionary analysis was conducted in MEGA11 (28). Study samples of the genogroup GII strains were associated with the GII.3 and GII.4 of the reference sequences.

4. Discussion

The main objective of the study was to investigate the presence and ecology of noroviruses along with the identification of the potential risk factors using the One Health approach by collecting samples from humans, animals, food, and beverages. Previous studies conducted in Pakistan had only investigated the noroviruses in humans, and this was the first study exploring the noroviruses in animals and food and beverages. The genetic diversity of the circulating strains in Pakistan was also identified through phylogenetic analysis by partial sequencing of three samples from humans. The strains found in our study were associated with GII.3 and GII.4 when compared with the reference sequences from the National Center for Biotechnology Information (NCBI) by the maximum likelihood method. Overall, the prevalence in humans for genogroup GII was 14% varying at the district level but there was no positive sample detected from bovines. From a wide range of food and beverage samples investigated, samples from sugarcane juice, tested in pools, were found positive for genogroup II (GII) and negative for genogroup III (GIII).

In humans, previous studies have reported a prevalence of 9.9% in Karachi alone, and 16.1% and 19.5% in studies conducted in multiple cities (8, 24, 29). Prevalence data reported in this study falls in the range of other studies conducted in similar settings such as 10.3% in India (30), 5.1% in Bangladesh (31), 11.2% in Taiwan (32), and 10% in Yemen (33). Many of the previous studies tested the GI and GII genogroups but in this study, we have only tested samples for the GII genogroup which is the most prevalent genogroup in humans at the global level including Pakistan (8, 31, 34, 35). A study conducted in the neighboring country, India, reported 82.5% of cases of genogroup GII as compared to 12.5% of cases for the GI genogroup (30). In most of the studies reported here, the target population was under 5 years of age and was hospital-based. Our study confirms the important role of norovirus infections as the cause of acute pediatric gastroenteritis in Pakistan.

The difference in the prevalence of disease between our study and the previous studies might be because they have studied both genogroups GI and GII, and we only studied GII to demonstrate that the GII genogroup is the most prevalent in Pakistan associated with noroviruses as confirmed by the various studied mentioned earlier. Most of the previous studies were also aimed to study other viruses but our study was purely designed to study noroviruses. They used real-time PCR, while we used the conventional PCR which is less sensitive as compared to the aforementioned. Due to limited resources, broad objectives of the study, and previous reports of the high prevalence of the GII genogroup, the study was designed to obtain an idea of the circulating virus through the One Health approach.

Through the phylogenetic analysis, the noroviruses detected in this study were genetically more associated with genogroups GII.3 and GII.4. From the risk factors analysis, previous direct contact with the acute gastroenteritis patient was a significant risk factor for infection which has also been reported by other studies (36, 37). The odds ratio reported in the previous study was 14.23 [95% CI: 6.5–31.0], and in our study, it was 3.7 [95% CI: 1.6–8.5]. Norovirus is very contagious and when a person comes in contact with an infected person while caring for them and/or sharing food or utensils which may have been contaminated by feces or vomit particles, there is a high chance that the other person will become infected (36). According to Centers for Disease Control and Prevention (CDC), infected individuals shed billions of norovirus particles and one can contract the infection by a few particles which can also be transmitted through the air.

In our study, the positive samples were detected more in female participants than in male participants, whereas a previous study conducted in Pakistan reported more prevalence in male participants than in female participants, which might be due to the larger sample size of the male participants compared to the female participants (8). In this study, the ratio of vomiting and fever presence was less as compared to the other studies, which shows the asymptomatic importance of norovirus infections (8).

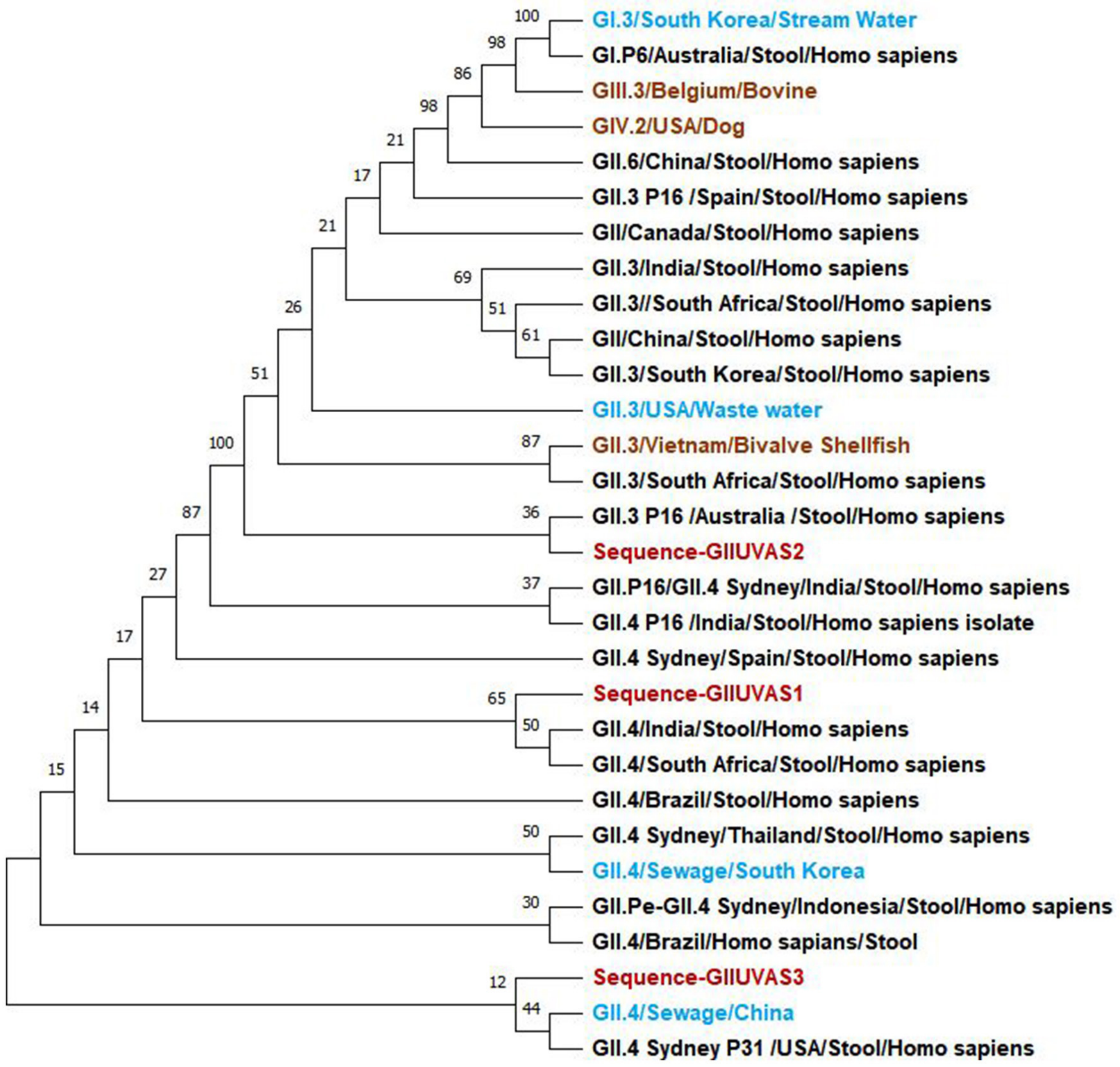

FIGURE 1
Phylogenetic relationships of norovirus GII genogroup isolates (red) from Pakistan compared to reference sequences in the NCBI database including the water (light blue), humans (black), and animals (brown) samples.

The risk factors evaluated in this study were selected based on findings from previous studies (36, 38), in which they were found to be associated with infection. While our study did not identify many statistically significant risk factors, but it is crucial that factors such as poor hand hygiene and consumption of contaminated water play a critical role in the transmission of noroviruses, particularly in low-income countries (37, 39). In this study, results were also non-significant for breastfeeding; although, in literature, it has a protective role against norovirus infection due to the norovirus-specific immunoglobulin A antibody in breast milk (40).

In our human study, several limitations can also be noted including the small sample size and selected study participants (hospitalized children under 5 years of age) which should be broadened in further studies as children may have a limited range of exposures in terms of food and other risk factors included in this study. We were also not able to sequence many samples due to budget constraints which need to be broadened to better understand the genetic variability of the norovirus strains circulating in Pakistan.

To our knowledge, this was the first study in Pakistan to investigate noroviruses in animals. Neonatal calf diarrhea is one of the main causes of economic loss in the livestock industry (41). According to the Economic Survey of Pakistan 2021–2022, livestock is contributing ∼14% to the national Gross Domestic Production (GDP) with more than 8 million rural families engaged in livestock production as their living source. Diarrhea is a frequent and growing concern leading to 24–63% of calf mortality in Pakistan (42). In Pakistan, the main etiological agents for calf diarrhea are rotavirus, coronavirus, enteropathogenic *E. coli*, Salmonella species, and Cryptosporidium, and most of the previous research is based on these agents (43, 44). Bovine norovirus has been proposed as one of the possible etiologies of calf diarrhea (45). They are also reported from the neighboring countries of Pakistan such as Iran and China (16, 46) which shows the threat of transboundary infection. Due to the ever-increasing trade in the livestock industry, now diseases are not limited to boundaries. The main objective of the study was to investigate the role of noroviruses in calf diarrhea as it is an emerging threat to the livestock industry worldwide. In future, further extensive investigation is recommended on a larger scale specifically during the calf diarrhea outbreaks to develop effective and realistic control and preventive strategies. From the 200 stool samples collected from the calves, there was no positive sample by RT-PCR. Many factors might be involved in the negative results which need to be addressed in future studies. First, no previous study was conducted on bovine noroviruses in Pakistan; hence, there might be a possibility that the virus may not be circulating despite reports of 20.4% prevalence in China and 39.5% in Iran which share borders with Pakistan (46, 47). Second, the sample size of our study was small (n=200), so there might be a possibility that the animals we screened were negative. This issue can be tackled by designing a nationwide study to assess the burden of noroviruses or can be combined with ongoing diarrheal illnesses' investigations.

Very little research has been conducted to explore the ecology of non-human populations. Close genetic relations between humans' and animals' noroviruses and the lack of information about the origin of new norovirus genotypes in humans have initiated the discussion about the possible routes for inter-species transmission (6). Up until now, there has been no evidence of the transmission of the virus from animals to humans; although, there are some serological hints observed in different studies for the possibility of transmission from animals to humans (48–50). In some studies, human noroviruses have been detected from animal stool samples (51, 52), and some experimental settings using animal models have also confirmed the ability to cross the species barrier (21, 53). To further investigate these findings, we adopted the One Health approach to better understand the relationship between norovirus ecology in humans and animals.

This study was also the first to investigate the presence of noroviruses in food and beverages in Pakistan. The sugarcane juice samples collected from local street food carts were found positive in our study. There can be multiple sources of contamination like the hands of the vendor and water or ice used. In our study, most of the food and beverage samples were negative, which is in accordance with food safety, but still, there is a risk of food contamination because the presence of noroviruses in humans is quite alarming and needs further investigation to assess the multiple risk factors associated with the disease. Surveillance, monitoring, and infection control system need to be strengthened for this purpose. However, some limitations can also be encountered in the food and beverages section of our study, such that we have analyzed samples in the pools, and the methods used for analysis are less sensitive.

Food and beverage products can be contaminated by fecal or vomitus material during the production process or by the hands of infected human beings (54). A study conducted by the Center for Food Safety, University of Georgia, to find out the transfer rate of norovirus from dry and wet stainless steel surfaces, found a transfer rate of more than 50% from wet surfaces as compared to dry ones. Depending on the type of water, NoV was able to persist in water for 60–728 days (55). Mostly in Pakistan, sugarcane juice machines are made of stainless steel and the surfaces remain wet all the time, so this might be a possible reason for the high persistent time for the virus to contaminate the sugarcane juice. Transmission from sugarcane juice can also be accompanied by multiple factors, including the contamination of ice (56), contaminated water usage for washing utensils, and using the same glass for many users (which is the most common practice in Pakistan) in which saliva can play an important role in the transmission of viruses (57). A similar study examining the presence of NoV in vegetables was performed by Cheong et al. However, only one spinach sample was found positive for NoV (58) which shows its low prevalence in vegetables which needs further investigation along with the large sample size enrollment and a better understanding of the diagnostic tests. Using conventional RT-PCR and manual extraction of viruses can be the other reasons for the no detection of noroviruses due to the low sensitivity of the processes.

5. Conclusion

This study concludes that noroviruses are contributing a significant part in childhood diarrhea cases with 14% positivity among the collected human diarrheal samples. All animal samples were negative. From the food and beverage samples, sugarcane juice samples were found positive with the recommendation to conduct further studies to roll out the associated risk factors with the noroviruses. This study provides an example of an interdisciplinary investigation of NoV at the human–animal–environment interface using the One Health principles; although little association was found in most of the risk factors, this study can be set as a pilot for the further investigation through the One Health approach which can provide much-needed insight into the prevalence and associated risk factors of norovirus infections.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. The animal study was reviewed and approved by Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing the original draft preparation, and project administration: AY and MH. Writing, reviewing, and editing: AY, YM, MY, U-u-RZ, CM-Z, MA, MW, and MH. Supervision: MH. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MA declared a shared affiliation with the authors AY, YM, and MY to the handling editor at the time of review.

Publisher's note

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Four years after the implementation of antimicrobial stewardship program in Jordan: evaluation of program's core elements

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Objectives: To combat antimicrobial resistance, the World Health Organization (WHO) urged healthcare organizations in Low- and Middle-Income Countries (LMICs) to implement the core elements of the antimicrobial stewardship (AMS) programs. In response, Jordan took action and developed a national antimicrobial resistance action plan (NAP) in 2017 and commenced the AMS program in all healthcare facilities. It is paramount to evaluate the efforts to implement the AMS programs and understand the challenges of implementing a sustainable and effective program, in Low-Middle Income Country (LMIC) contexts. Therefore, the aim of this study was to appraise the compliance of public hospitals in Jordan to the WHO core elements of effective AMS programs after 4years of commencement.

Methods: A cross-sectional study in public hospitals in Jordan, using the WHO AMS program core elements for LMICs was carried out. The questionnaire comprised 30 questions that covered the program's six core elements: leadership commitment, accountability and responsibility, AMS actions, education and training, monitoring, and evaluation, and reporting and feedback. A five-point Likert scale was employed for each question.

Results: A total of 27 public hospitals participated, with a response rate of 84.4%. Adherence to core elements ranged from (53%) in the leadership commitment domain to (72%) for AMS procedure application (actions). Based on the mean score, there was no significant difference between hospitals according to location, size, and specialty. The most neglected core elements that emerged as top priority areas were the provision of financial support, collaboration, access, as well as monitoring and evaluation.

Conclusion: The current results revealed significant shortcomings in the AMS program in public hospitals despite 4years of implementation and policy support. Most of the core elements of the AMS program were below average, which requires hospital leadership commitment, and multifaceted collaborative actions from the concerned stakeholders in Jordan.

KEYWORDS

antimicrobial resistance, antimicrobial stewardship program, WHO, low- and middle-income countries (LMICs), Jordan

Introduction

Antimicrobial resistance (AMR) is a significant threat to global health. At least 700, 000 deaths occur yearly due to AMR (1). The antimicrobial stewardship (AMS) program is one of the key strategies that has been proposed by the World Health Organization (WHO) Global Action Plan to solve the problem of the inappropriate use of antimicrobials and hence tackle AMR (2). The WHO, through the Global Action Plan on AMR, put five strategic objectives for countries as a guiding principle in developing their own AMR national action plans: The objectives focused on improving awareness and understanding of AMR, strengthening evidence-based knowledge through surveillance and research, reducing the incidence of infection, optimizing the use of antimicrobial medicines, and increase investment in new drugs (3).

In response to this public health threat, Jordan formed, in 2017, a multi-sectoral high-level committee headed by the Ministry of Health (MOH) that launched a 4 years national AMR action plan (NAP) in Jordan (2018–2022) (4). Fulfilling the NAP objectives, Jordan took the first steps in 2018 towards establishing a national AMR surveillance system with the aim of estimating the burden and describing the trend of AMR to inform the national treatment guidelines of prevalent infectious diseases, enhance infection prevention and control (IPC) programs to curb the spread of AMR, as well as to design appropriate AMS programs (5).

Antimicrobial resistance is a serious threat to global health. Jordan, part of the Eastern Mediterranean Region (EMR), has a high prevalence of self-medication with antibiotics; recently reported as 40% (6). In response to the global call, in 2017, Jordan launched the NAP and the implementation commenced. The national AMR surveillance activities started in October 2018. Jordan's AMR surveillance showed high resistance patterns of pathogens among inpatient and outpatient settings. High prevalence of extended spectrum beta-lactamase (ESBL) producers among *Escherichia coli* (47% in blood, 40% in urine) and *Klebsiella pneumonia* (51% in blood, 45% in urine) contributed to treatment failure using third-generation cephalosporins. This practice resulted in an increase in the number of prescriptions of carbapenems by physicians as a last resort in many complicated cases, which resulted in the emergence of carbapenem resistance which is more prominent in *Klebsiella pneumonia* (7–10).

Antimicrobial stewardship (AMS) is the optimization of the use of antimicrobials with the sole aim of improving patients' health outcomes and reducing AMR while avoiding unnecessary treatment costs. Designing AMS programs is way far from waving a magic wand to create a 'one-size fitsall' program, yet it is a set of different complementary strategies with a common ultimate target; to curb the problem of antibiotic resistance (11). AMS has evolved over the years and is more developed in high-income countries (HICs) compared to low- and middle-income countries (LMICs). Several factors affect the implementation of AMS programs in hospitals. The size and types of care provided, as well as the complexity of antibiotic prescription, are all issues that are considered in designing an effective hospital-based program (12). Taking the special context of LMICs, the WHO has identified in 2019 six core elements to evaluate the AMS programs at the facility level in LMICs, including: (1) Leadership commitment, (2) accountability and responsibility, (3) AMS actions, (4) education and training, (5) monitoring and evaluation, and (6) reporting and feedback (13).

It is paramount to conduct a contextual evaluation of the efforts to implement AMS programs. There are numerous challenges to implementing a sustainable and effective AMS program, in Low-Middle Income Country (LMIC) contexts. It is important to understand these challenges so that the stewardship initiatives can be tailored according to the unique requirements thrown up by these healthcare facilities (14). Consequently, there is an imminent need to gather sufficient data to evaluate the core elements of AMS programs at Jordanian hospitals. Thus, its time to take a more structured approach and evaluate the AMS program core elements in Jordanian hospitals after the release of the national AMR action plan based on WHO directions. Therefore, this research aimed at assessing the implementation status of the AMS program in public hospitals in Jordan. It also aims at identifying the key barriers and challenges to the implementation of AMS interventions.

Methods

Study design and study population

A cross-sectional study by means of a face-to-face interview was conducted between the 1st of April 2022 and the 30th of April 2022 with participants from the antimicrobial stewardship committees within the small, medium, and large public hospitals in Jordan. While public Hospitals with no established committee for antimicrobial stewardship were excluded. Therefore, 27 out of 32 public hospitals were approached. Private and Military hospitals were excluded due to the lengthy process to obtain approvals and the limited timeframe to conduct the research.

The primary data source was derived from the questionnaires filled in by the focal members of antimicrobial stewardship committees within the participating hospitals. The inclusion criteria comprised the small, medium, and large hospitals, as accredited and non-accredited hospitals within the public healthcare sector. Primary healthcare centers and ambulatory clinics were excluded.

The WHO Questionnaire was used as the research tool. It was distributed to 27 public hospitals that had an AMR committee, and a representative of the committee was interviewed and delegated to answer the questionnaire on behalf of the hospital.

TABLE 1 Demographic characteristics of participants.

Demographic parameter	Overall (*n* =27) Frequency (%)
Educational level	
Bachelor's degree	18 (66.7)
Postgraduate MSc	9 (33.3)
Gender	
Female	23 (85.2)
Level of experience	
1–5 years	1 (3.7)
6–10 years	9 (33.3)
11–15 years	13 (48.1)
More than 15 years	4 (14.8)
Position	
Pharmacist	12 (44.4)
Clinical Pharmacist	15 (55.6)

Sampling strategy

A total of 27 out of the 32 public hospitals were invited to participate in the study, while 5 were excluded due to the lack of an established AMS committee. Three investigators were assigned to contact the management in each hospital through the primary investigator. Each investigator was assigned a group of hospitals and interviewed a representative of the AMS committee in each hospital. In order to unify the interview approach and to exclude any bias, the three investigators met and discussed the questions and agreed upon the description of each question as per the guidance included within the WHO tool.

During the interview, the questionnaire was read and explained to each participant. The explanation was based on the detailed description included within the WHO tool. All participants voluntarily participated in the study and were thus considered exempt from written informed consent, which was written at the beginning of the tool before starting. The study's aim and objectives were clearly explained at the beginning of the survey questionnaire.

The questionnaire

The questionnaire was made up of three sections. The first section focused on participants' background and demographic information (age, gender, educational level, and job position). The second section entailed questions that described and categorized the characteristics of the hospital (sector, geographic location, hospital size, accreditation status), and the scope of services (general, specialized). The third section dealt with the availability of AMS program core elements. In this section, a five-point Likert scale was employed, and 30 questions were further divided into six focus domains (Supplementary material). The domains are: (1) Leadership commitment, (2) accountability and responsibility, (3) AMS actions, (4) education and training, (5) monitoring and evaluation, and (6) reporting and feedback. The questionnaire was adopted from the WHO practical toolkit for antimicrobial stewardship programs in healthcare facilities in low- and middle-income countries (2019) (13). The tool asked the participants about the degree to which he/she agrees with the level of implementation and availability of each question within the six core elements. The participants' responses ranged from 1 to 5, and the average score for each area was calculated, as well as the weighted average for each question.

Ethical approval

All study participants gave their informed consent for inclusion before they participated in the study. The study protocol was approved by the Ministry of Health in Jordan (IRB approval no. 2232).

Statistical analysis

Data was analyzed employing SPSS software, version 25. Descriptive statistics were used to describe the participants' demographic characteristics. Data were reported as mean ± SD for normally distributed variables, while categorical data were reported as percentages (frequencies). Cronbach's alpha was employed to measure the reliability of the tool with a set of 0.96 as the scale of reliability. Two-way ANOVA was employed to measure significance with a significance level set at 0.05.

Results

Background and demographics

A total of 27 healthcare providers representing the AMS committee participated in the study from 27 hospitals. Table 1 details the baseline characteristics of the participants. The majority of participants were females (85.2%) and bachelor's degree holders (66.7%). When it comes to the experience of participants, 96% of them have more than 5 years of experience. All AMS committee focal points were either pharmacists (44.4%) or clinical pharmacists (55.6%).

Table 2 presents the distribution of participants according to the demographics of their organizations. The majority of hospitals were in the middle region of Jordan (48.1%). Moreover, 19 (70.4%) hospitals were with be capacity ranging from 100 beds to 500 beds. In terms of scope of services, 21 (77.8%) were general hospitals. With regards to accreditation status, the hospitals were almost equally distributed among accredited (51.9%) and non-accredited (48.1%) hospitals.

Responses according to AMS program core elements

The assessment tool consisted of 30 questions based on the WHO's six core domains of the AMS program. The results of AMS core elements application for all hospitals are showed in Table 3. The cut-off point was estimated by using the quartile percentile; P25 = **2.75**, P50 = **3.10**, and P75 = **3.60**. Therefore, the cut-off point needed to assume a good level of AMS application in the hospital was considered as P50 (**3.10**). Generally, most of the domains showed low application levels (below **3.10** out of **5**); the highest level was in AMS procedure

TABLE 2 Participants' hospitals characteristics (the distribution was based on participants responses).

Domain	Overall (*n*=27) Number (%)
Hospital size	
Small (less than 100 beds)	7 (25.9)
Medium (100–500 beds)	19 (70.4)
Large (more than 500 beds)	1 (3.7)
Region of the hospital	
North	11 (40.7)
Middle	13 (48.1)
South	3 (11.1)
Accreditation status	
Accredited	14 (51.9)
Non-accredited	13 (48.1)
Scope of service	
General hospital	21 (77.8)
Specialized hospital	6 (22.2)

TABLE 3 The overall application average of antimicrobial stewardship (AMS) six core elements.

Domain	Application average (%)
Leadership commitment	2.66 (53)
Accountability and responsibilities	3.15 (63)
AMS actions	3.61 (72)
Education and training	2.85 (57)
Monitoring and surveillance	3.01 (60)
Reporting and feedback	2.96 (59)

application (actions; **3.61**), and the lowest application was in leadership commitment (**2.66**).

Additionally, the correlation was used to explore the relationship between the availability of AMS core elements and other hospital characteristics such as capacity (large, medium, small), location (north, center, and south), the scope of work (general or specialized), or if the hospital was accredited or not. Generally, the analysis showed that there were no statistical correlations between the availability of core elements for the AMS program and these variables and there was no significant difference among those variables.

Table 4 details the level of application of each question under each core domain. Within the first core domain **(leadership Commitment)**, the highest application within hospitals was for having an AMS action plan (65.86%). While prioritizing the AMS program scored (59.2%) and dedicating financial support scored the lowest (34.8%). For the second core element **(Accountability and Responsibility)**, results showed a score of 74% among participating hospitals for the availability of a multidisciplinary AMS leadership committee with clear terms of reference. However, the presence of clearly defined collaboration between the AMS and IPC programs scored low (51.8%). The availability of **AMS actions** was the highest-scoring core element among the six AMS areas (72%). Results showed that almost all participating hospitals have a formulary with a list of approved antibiotics. When it comes to **Education and Training,** the overall compliance was low (57%) among all participating hospitals. Provision of basic training in optimal antibiotic use for healthcare professionals scored only (48.84%). Looking at the availability of the **Monitoring and Evaluation** core element, results showed that the monitoring of antibiotic susceptibility and resistance rates was scored below average (59.2%). As for the monitoring of the quantity and types of antibiotic use at the unit and/or facility-wide level, it was poorly applied scoring 67.34%. The analysis of the **Reporting and Feedback** element showed that regular evaluation and sharing of resistance rates were poorly conducted with a score of 56.98%. Developing an antibiogram for key antibiotics attained a low score that did not exceed 59.2%.

Discussion

This study explored the current status of the implementation of the AMS program in public hospitals in Jordan. Overall, 84.4% of the public-hospital (27 out of 32) established an AMS committee, which was the target of this study. Using the WHO's core elements, we found that compliance ranged from 52 to 72%, and hospitals reported having an AMS program that implemented all the core elements defined by the WHO in 2018 in response to the national action plan (4). Four years after starting the implementation of the NAP, the results of the national AMR surveillance program reflect alarmingly rising levels of multidrug-resistant pathogens (15). The findings of the surveillance reports clearly revealed the scale of the problem, yet the effectiveness of solutions and interventions that have been put in place 4 years ago is not clearly demonstrated.

In 2017, the implementation of the AMS program core elements, as aligned by the CDC, was evaluated in Jordanian Hospitals. Ababneh et al. conducted a cross-sectional study in 41 Jordanian hospitals regarding adherence to the CDC criteria for the AMS program. Among the enrolled hospitals in the study, 17.1% had an infectious diseases specialist as the head of AMS, whereas 73.2% had clinical pharmacists involved as AMS leaders (16). In comparison, the results of the current study revealed interesting findings pertinent to the structure of the AMS committee. All public hospitals had either a pharmacist or clinical pharmacist leading the AMS program. Despite variations among countries in terms of AMS committee structure, the consensus is that pharmacists/ clinical pharmacists are a fundamental part of the committee (17–19). Their role can entail developing and managing guidelines, education, monitoring compliance with antimicrobial use and auditing outcome of use (18, 20, 21). However, the role of pharmacists in Jordan was explored due to the shortage of medical professional expertise. Several studies reported that pharmacists expressed difficulties conveying their opinions and recommendations on antibiotic therapy to physicians despite frequent communications (22). Thereover, it is vital to empower pharmacists to lead AMS interventions and drive change.

The findings in this study showed an alarming result when it relates to leadership commitment, particularly in terms of the provision of financial support with a total score of 34.8%. In fact, AMS programs in hospitals need significant funding support, trained human resource, and political will (23–25). A robust level of implementation of stewardship measures in a hospital requires a

TABLE 4 Participants' responses regarding the AMS core elements.

No.	Domain	Strongly disagree frequency (%)	Disagree frequency (%)	Do not know frequency (%)	Agree frequency (%)	Strongly agree frequency (%)	Total score (%)
	Leadership commitment						
1.1.	AMS program is identified as a priority for health-care facility management	4 (14.8)	5 (18.5)	7 (25.9)	10 (37.0)	1 (3.7)	59.2
1.2.	Health-care facility has AMS action plan that prioritizes activities and measures progress and accountability	5 (18.5)	3 (11.1)	2 (7.4)	13 (48.1)	4 (14.8)	65.86
1.3.	There are dedicated financial support for the health-care facility AMS action plan	16 (59.3)	4 (14.8)	5 (18.5)	2 (7.4)	0 (0)	34.8
2.	Accountability and responsibility						
2.1.	A multidisciplinary AMS leadership committee in place with clear terms of reference	3 (11.1)	3 (11.1)	3 (11.1)	8 (29.6)	10 (37.0)	74
2.2.	A dedicated AMS leader/champion is identified within the health-care facility	5 (18.5)	2 (7.4)	6 (22.2)	8 (29.6)	6 (22.2)	65.86
2.3.	The multidisciplinary AMS team is in place with terms of reference	4 (14.8)	3 (11.1)	9 (33.3)	6 (22.2)	5 (18.5)	63.64
2.4.	Other health professionals are identified and involved in AMS activities	5 (18.5)	4 (14.8)	3 (11.1)	14 (51.9)	1 (3.7)	61.96
2.5.	There is a clearly defined collaboration between the AMS and IPC programs	6 (22.2)	7 (25.9)	7 (25.9)	6 (22.2)	1 (3.7)	51.80
2.6.	There are regular (descriptive) activity reports on the implementation of the AMS program	4 (14.8)	7 (25.9)	5 (18.5)	5 (18.5)	6 (22.2)	61.42
3.	AMS actions						
3.1	There are up-to-date standard treatment guidelines	4 (14.8)	1 (3.7)	5 (18.5)	13 (48.1)	4 (14.8)	68.82

(Continued)

TABLE 4 (Continued)

No.	Domain	Strongly disagree frequency (%)	Disagree frequency (%)	Do not know frequency (%)	Agree frequency (%)	Strongly agree frequency (%)	Total score (%)
3.2.	Regular AMS team review/audit of specified antibiotic therapy or clinical conditions is conducted at the healthcare facility	2 (7.4)	1 (3.7)	9 (33.3)	13 (48.1)	2 (7.4)	68.82
3.3.	Advice/feedback from AMS team members is easily accessible/ available to all prescribers	3 (11.1)	1 (3.7)	2 (7.4)	17 (63)	4 (14.8)	73.34
3.4.	The AMS team conducts regular ward rounds and other AMS interventions in select health-care facility departments	3 (11.1)	2 (7.4)	6 (22.2)	16 (59.3)	0 (0)	65.94
3.5.	Health-care facility has a formulary with a list of approved antibiotics	1 (3.7)	1 (3.7)	1 (3.7)	11 (40.7)	13 (48.1)	85.1
3.6.	Health-care facility has a list of restricted antibiotics	2 (7.4)	3 (11.1)	2 (7.4)	10 (37)	10 (37)	76.96
3.7.	Laboratory and imaging services are accessible to support AMS interventions	6 (22.2)	2 (7.4)	7 (25.9)	8 (29.6)	4 (14.8)	61.42
3.8.	Health-care facility has access to IT services to support AMS activities	5 (18.5)	4 (14.8)	7 (25.9)	9 (33.3)	2 (7.4)	59.2
3.9.	There is a standardized facility prescription chart and medical records	0 (0)	2 (7.4)	8 (29.6)	17 (63.0)	0 (0)	71.12
3.10.	There is a Health-care facility policy for documenting prescribed medicines	1 (3.7)	4 (14.8)	1 (3.7)	17 (63.0)	4 (14.8)	74.08
4	Education and training						
4.1.	Basic training in optimal antibiotic use is provided for health-care professionals	7 (25.9)	9 (33.3)	5 (18.5)	4 (14.8)	2 (7.4)	48.84

(Continued)

TABLE 4 (Continued)

No.	Domain	Strongly disagree frequency (%)	Disagree frequency (%)	Do not know frequency (%)	Agree frequency (%)	Strongly agree frequency (%)	Total score (%)
4.2.	Continued training in optimal antibiotic use is provided for health-care professionals	7 (25.9)	9 (33.3)	5 (18.5)	5 (18.5)	1 (3.7)	48.1
4.3.	Initial and regular training of the AMS team in infection management is provided	5 (18.5)	9 (33.3)	8 (29.6)	5 (18.5)	0 (0)	49.58
5	Monitoring and evaluation						
5.1.	There is monitoring of the appropriateness of antibiotic use at the unit and/or facility-wide level through audits or PPSsi	2 (7.4)	7 (25.9)	8 (29.6)	7 (25.9)	3 (11.1)	61.42
5.2.	There is monitoring of the quantity and types of antibiotic use (purchased/ prescribed/dispensed) at the unit and/or facility-wide level	2 (7.4)	4 (14.8)	8 (29.6)	8 (29.6)	5 (18.5)	67.34
5.3.	There is monitoring of antibiotic susceptibility and resistance rates for a range of key indicator bacteria	6 (22.2)	4 (14.8)	4 (14.8)	11 (40.7)	2 (7.4)	59.2
5.4.	There is monitoring of the compliance with AMS interventions by the AMS committee	4 (14.8)	5 (18.5)	7 (25.9)	10 (37.0)	1 (3.7)	59.2
6	Reporting and feedback						
6.1.	Regular evaluation and sharing of health-care facility data on antibiotic use with prescribers is done	4 (14.8)	4 (14.8)	6 (22.2)	10 (37.0)	3 (11.1)	63.54
6.2.	Regular evaluation and sharing of health-care facility resistance rates with prescribers is done	5 (18.5)	3 (11.1)	13 (48.1)	3 (11.1)	3 (11.1)	56.98

(Continued)

TABLE 4 (Continued)

No.	Domain	Strongly disagree frequency (%)	Disagree frequency (%)	Do not know frequency (%)	Agree frequency (%)	Strongly agree frequency (%)	Total score (%)
6.3.	Evaluation of appropriateness of data on antibiotic use is shared with prescribers	4 (14.8)	5 (18.5)	8 (29.6)	5 (18.5)	5 (18.5)	61.42
6.4.	Health-care facility develops antibiogram for key antibiotics informed by data on antibiotic use and resistance	5 (18.5)	5 (18.5)	5 (18.5)	10 (37.0)	2 (7.4)	59.2

committed team of experts; and the support of microbiology laboratories and hospital information systems (26). In the context of Jordanian hospitals, very little effort has been taken to look at the feasibility of implementing AMS interventions (27). The interventions that are feasible and effective in low-resource settings, may be different from those which has succeeded in larger hospitals situated in high-income countries (14). Similar findings were reported as a key challenge to effective AMS programs (28, 29) even in high-income countries like the United States (30). A study in Indonesia reported that less than 50% of hospitals allocated funds to support AMS programs (28). A multinational group of experts among EU and US hospitals agreed on core indicators to assess the AMS programs, financial support to the provision of salary funding for dedicated support of AMS activities was considered a core indicator (30). Similar results were also reported in the study of Ababneh et al. (16) where none of the assessed facilities presented a financial report endorsing ASP responsibilities.

Two encouraging findings of this study are associated with the two key questions in the action domain, which received the highest mean score: the availability of a formulary with a list of approved antibiotics (85.1%) and the availability of an approved list of restricted antibiotics (76.96%). However, the lack of adequate audit (68.82%) prevents the feasible evaluation of the effectiveness of these processes. Unfortunately, the antibiotic restriction may not stop the possible overuse of existing broad-spectrum antimicrobials (31). The availability of structure indicators is vital in AMS programs, however, process and outcome indicators of the program were not encouraging (16, 32). Particularly if that was not accompanied by continuous education and training (32, 33).

In this study, education and training scored below average (57%). Literature also supports that investment in education and training can substantially improve the outcome (23–25, 30, 34). The next logical step is to translate commitment and education into actions. Several studies reported that the main source of the misuse of antimicrobials is a lack of knowledge among healthcare professionals (11, 13, 34). A cross-sectional study in Jordan in 2021, focused on identifying the perceptions and practices among Jordanian healthcare practitioners toward AMS programs. The findings revealed a positive perception towards the program, while practices pertinent to this element were suboptimal. Further, the results showed that longer years of practice, postgraduate studies, and practice in academia sectors yielded higher perception scores (p value = 0.0335, 0.0328, and 0.0007 respectively) (35). Therefore, it is now vital that both academia and the MOH cooperatively focus on integrating antimicrobial resistance and good practices in all healthcare professional curricula and in-service educational sessions (34). Such educational sessions need also to include community awareness. A study by Alzoubi and coworkers (36), reported a low average knowledge about the use of antibiotics among 1,091 Jordanian patients attending outpatient clinics. Only 20.1% of the participants stated that antibiotics were used for bacterial infections. Moreover, several studies revealed that the prevalence of self-medication with antibiotics in Jordan remains high (37, 38), this in turn counteracts efforts of AMS in hospitals and leads to them being ineffective. Enforcement of legislation may need to be pursued to enable successful AMS programs in Jordan.

Another prominent challenge that was identified in this study was the lack of collaboration. This was evident from the answers to the following question "*There is a clearly defined collaboration between the AMS and IPC programs*" (score 51.8%). Effective AMS programs required a multifaceted collaboration within the organization and even outside it (39). Collaboration between IPC and AMS committees can enhance performance between the two parties, produce synergistic actions and mitigate any impediments (25, 40). Collaboration between the two committees has it rational as both serve a common purpose and they share similar expertise. The earlier study in Jordan (16) reported that the personnel who were most collaborative with the AMS team were clinicians (51.2%) and the least collaborative were microbiologists (17.1%). Therefore, this lack of collaboration will impact the effectiveness of the AMS program.

Access to IT services, laboratory, and imaging services to support AMS activities were scored low, 61.42, and 59.20%, respectively. Delays in service provision are critical for effective clinical outcome. Several reports identified the importance of timely intervention and that the reduction in the use of antibiotics is not the key attribute, reduction of inappropriate use and timely access to effective treatment is the key indicator for effective AMS programs (24, 41). Limited access imparts timeliness to the provision of care. Effective AMS program requires regular updates on relevant information that may include resistant bacteria, the incidence of antibiotic-resistant bacteria which requires easy access to IT and laboratory services (42). The IT services need to be integrated within the AMS program due to their role in the provision of support for decisions concerning antibiotic prescriptions, offering facilities for the collection and reporting of antibiotic use, as well as providing information and protocols that can be directly linked to AMS program or clinical guidelines, hence, possibly improving the rational use of antimicrobials (28, 43). An example of the use of technology that supported AMS programs is the implementation of automatic stop order (ASO), which is considered one of the technological tools by which identified medications are re-evaluated and reviewed on a consistent basis to ensure preventing unreasonable and prolonged use of drugs. With ASO, stop dates are automatically applied to an antimicrobial order when the duration of therapy is not specified. The goal is to ensure that antimicrobials are continued no longer than necessary. ASO encourages reassessment of the duration of therapy based on the patient's response to treatment, and prescriber review of laboratory, microbiology and diagnostic imaging results after the specified length of time (44). In Jordan, the use of ASO is in its infancy.

Monitoring and evaluation (overall score of 60%), as well as reporting and feedback (overall score 59%) were also scored low in this study. Jordan public hospitals are still in the early stages of implementation and a lot of efforts need to be made to comply with such aspects. In comparison with other countries, A study in acute care hospitals in the United States reported that 79.3% of surveyed hospitals complied with monitoring of prescribing and antibiotic resistance patterns (30). Several countries employed pharmacists and clinical pharmacists to lead the monitoring and evaluation processes of the AMS programs (17–19). In a study in India, pharmacist-led model over 1 year resulted in an increase in prescribed antibiotic appropriateness from 56 to 80% and compliance to recommendations increased from 54 to 70% (21). In reference to that, as pharmacists/clinical pharmacists are leading the AMS program in Jordan, it is proposed that they are empowered to lead the monitoring and evaluation processes.

Implication of the study

The WHO core elements for the assessment of the effectiveness of AMS programs needs to be employed to conduct periodic cross-sectional audits for the public and private sector in Jordan. This will enable tracking the progress of the implementation of the program across the country and identifying gaps within the practice. Jordan has made commitments to curb antimicrobial resistance using the NAP (4). Nevertheless, the findings of this study raise apprehensions over the implementation gaps in rendering the political commitment of the MOH into favorable actions. Consequently, the findings of this study set as a reminder to accelerate the implementation of NAP that entails AMS programs in various healthcare settings in Jordan and other LMICs. There is a need to expand training and professional education on AMR, MoH could consider focusing efforts on physicians. A recent study found that one-third of physicians reported no knowledge of any initiatives on antibiotic awareness and resistance and nearly 90% were unaware or unsure of the existence of a NAP on AMR (45). Additionally, IPC compliance and effectiveness needs to be regularly assessed in health facilities. Further, sufficient and consistent financing is key to the sustainable implementation of AMR actions in Jordan, therefore budget allocation for the implementation of AMS program is needed within each hospital.

Additionally, it is important to further explore barriers and facilitators of implementing interventions to improve antimicrobial stewardship in Jordan through a qualitative study to investigate the real implementation status of AMS program. A study reported that in 2019, approximately 59% of the antibiotics consumed nationally were from the WHO's AWaRe classification of antibiotics (46, 47). Therefore, future study needs to consider evaluating the class of antibiotic usage.

Strengths and limitations

To the best of our knowledge, this is the first study in Jordan that assess the AMS program after 4 years of the implementation of the NAP in 2017 using the WHO core elements for the AMS program for LMIC. There is a limited number of publications in LMIC assessing the effectiveness of the AMS program. However, this study had a few limitations. First, the use of self-reported data is subject to bias. Some of the participants may have overestimated or underestimated their responses to the questionnaire, impacting the accuracy of the findings. Second, the military and private hospitals were not included and therefore, the generalizability of the results is not feasible. Third, no pre-implementation data were available to attribute the observed results to the AMS program. Despite that, in terms of impact, the study described specific gaps in the AMS program in public hospitals in Jordan. These results are similarly useful for other hospitals in Jordan (private or military) as well as other LMICs. An additional limitation is pertinent to the limited number of studies that evaluated AMS programs in LMICs using the WHO core elements, particularly in the middle east, which limited our ability to compare our findings with similar programs. Owing to the study design (questionnaire with closed-end answers), we might have missed some information that could be captured using open end questions.

Conclusion

The current study elaborated on the level of implementation of the AMS program in public hospitals in Jordan, using the WHO core elements for the evaluation of the AMS program at the facility level in LMICs. Overall, the level of implementation of the program was not optimal and there is still much more that needs to be done. Key gaps were identified pertinent to the provision of financial support, lack of training, poor collaboration, and inadequate access to IT and diagnostic services. A well-structured monitoring, and evaluation processes as well as feedback provision were below the average. Leadership and key stakeholders' commitment and support underpin the success of the AMS program. Overall, the results provided a baseline to monitor progress toward the national AMR action plan (NAP) in Jordan.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

All study participants gave their informed consent for inclusion before they participated in the study. The study protocol was approved by the Ministry of Health in Jordan (IRB approval no.2232).

Author contributions

SH: conceived the study and project management. ED: led writing, reviewing and editing the manuscript, and validation of results. TM: conceptualization and project supervision. OT: implementation of the research and statistical analysis. TJ and WA: data collection and implementation of the research. RG: study design and implementation of the research. ZH: implementation of the research. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1078596/full#supplementary-material

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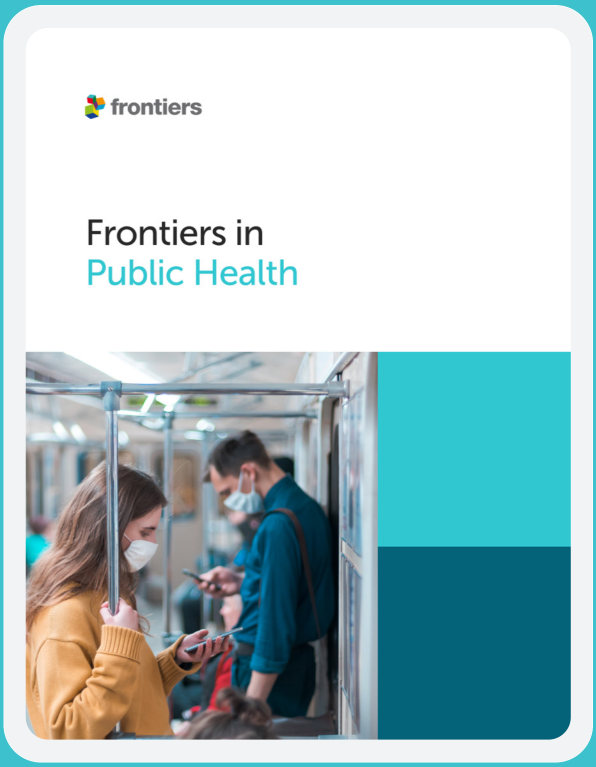

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